COMMUNITY ASSEMBLY OF NEOTROPICAL FROGS ACROSS ECOLOGICAL SCALES

A Dissertation

by

CHRISTOPHER MICHAEL SCHALK

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Chair of Committee,	Lee A. Fitzgerald
Committee Members,	Thomas E. Lacher
	Amanda Stronza
	Kirk O. Winemiller
Head of Department,	Michael Masser

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ABSTRACT

Ecological communities are organized by historical, biotic, and abiotic factors and the strengths of these factors vary across multiple spatial and temporal scales. I sought to disentangle the drivers of community assembly in frogs of the Bolivian Gran Chaco. This research was conducted within the framework of Applied Biodiversity Science that incorporates collaborations with local institutions to achieve biodiversity conservation. I used functional traits to examine the relationship between species distribution in niche space and community diversity of post-metamorphic frogs. Community organization was non-random, and species-packing significantly increased with increasing community diversity, a pattern indicative of response to environmental filters. I quantified the spatiotemporal dynamics of pond food webs and observed that consumers were trophic generalists, with many species occupying more than one trophic position. Breeding ponds are patchy and ephemeral across the landscape, and being trophic generalists enables consumers to exploit various resources within a pond. I conducted an experiment to examine predator-induced plastic responses in tadpoles of an arid-adapted species, *Leptodactylus bufonius*, a species with terrestrial oviposition and aquatic development. Tadpoles in the predator treatments exhibited the strongest responses when exposed to both predator and conspecific alarm cues. Species adapted to breed in ephemeral ponds with terrestrial oviposition may be able to afford reduced growth rates by capitalizing on a head start in development. I examined the relationships between calling activity and environmental factors. Calling phenology of tropical

anurans is the product of exogenous factors (i.e., climatic variables) and endogenous factors (i.e., reproductive modes). I also studied intra-specific variation in ambush site selection in metamorphs and adults of *Ceratophrys cranwelli*. Metamorphs selected a subset of available habitat, whereas adult frogs did not appear to select ambush sites based on variables I measured. This study provides insights to the trade-offs between foraging strategy and mortality risk. A synthesis of the diet in frogs of the Family Ceratophryidae revealed they are generalist, opportunistic predators. Contrary to previously published literature, there is little evidence of larval cannibalism in these species. The results of my dissertation provide important biological information useful in developing conservation strategies needed for habitat management and biodiversity conservation.

DEDICATION

To my family for sparking my interest in the natural world. And to my wife, Carmen, whose support is a constant source of inspiration in maintaining that spark.

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CHAPTER I

INTRODUCTION

The world is experiencing a loss of biodiversity that greatly exceeds the background extinction rate (Ceballos *et al.* 2015). While some argue biodiversity can only be protected through strict enforcement in protected areas where people are absent (Terborgh 1999), others have argued that landscapes have never been devoid of people (Cronon 1996) and conservation strategies that do not include local people are not only ineffective, but likely to fail (Adams & McShane 1992). While park-based models have been advocated as being the most effective method to preserve biodiversity (Terborgh 1999), the establishment of partnerships between conservation and local indigenous groups have been demonstrated to be successful (Jamal & Getz 1995; Arambiza & Painter 2006; Redford & Painter 2006; Brightsmith *et al.* 2008; Mbaiwa & Stronza 2011). However, in forging partnerships, the motivations with all of the actors involved must be explicitly stated, as each groups' motivations can differ fundamentally (Redford & Painter 2006). The framework of Applied Biodiversity Science seeks to integrate biodiversity research while the broader impacts of these research efforts be applied to meeting on-the-ground conservation objectives (Fitzgerald & Stronza, 2009). Applied Biodiversity Science recognizes the need to integrate both biological and social sciences while working with local actors and institutions to apply conservation theory to implement effective conservation strategies (Fitzgerald & Stronza, 2009).

The Gran Chaco, an immense biogeographical spanning the countries of Argentina, Bolivia Brazil, and Paraguay, is comprised of a diverse mosaic of habitat types, such as thorn forest and palm savannahs. Deforestation rates of the Chaco have been among the highest in the world (Zak *et al.* 2004), with much of the land being cleared in Argentina and Paraguay for agricultural and ranching efforts. The Chaco region in Bolivia has been one of the last strongholds of relatively undisturbed Chaco habitat compared to the neighboring countries of Argentina and Paraguay. Recognizing the value of the Bolivian Chaco for conservation, The Wildlife Conservation Society (WCS) established a partnership with the Capitania de Alto y Bajo Izozog (CABI) with a central goal of conservation of the Bolivian Gran Chaco. CABI is an indigenous organization that represents the Guarani people living in 27 communities along the Parapetí River in the Bolivian Chaco. The Wildlife Conservation Society saw the value in the landscape of the Gran Chaco as an underappreciated biodiversity hotspot, particularly for mammals (Redford *et al.* 1990). The indigenous Isoceño Guaraní people saw an encroaching threat of Mennonite colonists beginning to purchase and covert large areas of the Chaco for dairy production and large-scale farming (Taber *et al.* 1997).

Conservation programs in the Gran Chaco are an excellent model for community-based conservation. The institutions of both WCS and CABI were integral in the establishment of the Kaa-Iya del Gran Chaco National Park and Integrated Management Area in 1995 (Taber *et al.* 1997). At the end of the process, over 5.3 million hectares were protected under the new park as well as securing a title to their territory (Taber *et al.* 1997). An additional success was CABI being allowed to co-

manage the Kaa-Iya Park with the Bolivian government, resulting in the largest and only park co-managed by an indigenous group in the Americas (Taber *et al.* 1997). Other examples of co-management, such as in community-based ecotourism have also been successful (Brightsmith *et al.* 2008; Stronza & Pegas 2008). My dissertation research was conducted in this setting of indigenous communities, protected areas, and territorial lands, with the objective that results from my dissertation complement current biodiversity research with the national government, indigenous people, NGOs, and local institutions to develop integrative conservation strategies.

Amphibians are one such group that is experiencing steep population declines; nearly one-third of all amphibian species are threatened with extinction (Stuart *et al.* 2004) and currently exceed the background extinction rate by over 200 fold (McCallum 2007). The drivers of amphibian declines are complex and not mutually exclusive from one other and include habitat loss, climate change, invasive species, and pathogens (Kiesecker *et al.* 2001). Amphibians can constitute a significant portion of the animal biomass in some ecosystems (Gibbons *et al.* 2006) and serve as important linkages in food webs as both predators (Wells 2007) and prey (Toledo *et al.* 2007). Thus, understanding the patterns and mechanisms that drive community assembly in amphibians can have important implications for their role in the broader context of ecosystem structure and function (Wells 2007). This is further complicated by the fact that most amphibians possess a complex life cycle (Wilbur 1980) as different factors are important in the regulation and structure of each life stage, representing a particular challenge for conservation and management (Semlitsch 2000).

The WCS worked closely with CABI and the people of Isoso in training and building the capacity of 20 parabiologists to monitor and study the local biodiversity in Isoso and the management areas bordering the core area of Kaa-Iya National Park. The program was the largest and most effective wildlife research program in the Gran Chaco and an excellent model for any such program (Noss & Painter 2004; Noss *et al.* 2005). These efforts include estimating abundance, habitat use, and resources of species of conservation importance in line with WCS objectives, such as jaguars (*Panthera onca*; Maffei *et al.* 2004) as well as species of importance to the livelihoods of the Isoceño people such as game animals and red tegu lizards whose skins are sold to local tanneries for supplemental income (Cuéllar *et al.* 2010; Montaño *et al.* 2013a, b; Noss *et al.* 2013). Programs emerging from these efforts to include the entire community include a self-monitoring program involving hunters and wildlife monitors (Noss *et al.* 2005).

These conservation and capacity building efforts in the Bolivian Chaco have predominately focused on terrestrial biodiversity. In conjunction with my dissertation research, I sought to complement the efforts of WCS and CABI by expanding the capacity of the Isoceño parabiologists and park guards to continue their environmental stewardship of the area of Isoso and the Kaa-Iya National Park. Specifically, I worked together with the parabiologists and park guards to develop skills and build their capacity in measuring and monitoring biodiversity primarily associated with aquatic habitats, including amphibians. Prior research on the frogs of the Bolivian Gran Chaco has focused on documenting species diversity and distributions (Gonzales 1998; Gonzales et al. 2006; Montaño & Gonzales 2008). However, ecological communities are

structured as a result of local and regional processes that vary across time (Ricklefs & Schluter 1993). My dissertation research built upon these previous efforts of Bolivian scientists by focusing on examining the patterns and mechanisms of coexistence across multiple spatial and temporal scales of both larval and post-metamorphic anuran amphibians in the Bolivian Gran Chaco. I sought to examine the relationship between community diversity and functional traits to elucidate the factors that allow the coexistence of post-metamorphic anurans (Chapter II). I examined the abiotic factors that drive calling phenology in anurans to understand the temporal community structure as the breeding phenology can have consequences for community structure of larvae within the breeding ponds (Chapter III). I also examined the determinants of food web structure along environmental gradients of the breeding ponds utilized by these species (Chapter IV). I conducted a laboratory experiment to understand how tadpoles are able to coexist with predators by examining their plastic responses of behavior and morphology (Chapter V) and conducted a study to quantify the intra-specific variation in niche partitioning of habitat selection in a Gran Chaco endemic, the Chacoan Horned Frog (*Ceratophrys cranwelli*) (Chapter VI). To better understand the resources utilized by Ceratophryid frogs, a family of frogs that is notable because many species are predators on other frogs and tadpoles, I reviewed feeding habitats of all species in the family Ceratophryidae (Chapters VII). Overall, the research conducted as part of this dissertation seek to better our understanding on the mechanisms of community assembly across multiple spatial and temporal scales.

CHAPTER II

SPECIES PACKING, FUNCTIONAL TRAITS, AND COMMUNITY ORGANIZATION OF NEOTROPICAL FROG ASSEMBLAGES

Introduction

Ecological communities are structured as the result of the interaction between local and regional processes (Ricklefs 1987; Cornell & Lawton 1992; Ricklefs & Schluter 1993; Mittelbach & Schemske 2015). Regional processes, such as species-specific responses to abiotic factors, and distributional constraints set by historical biogeography tend to exert stronger influence at broad spatial scales (Ricklefs & Schluter 1993; Ryberg & Fitzgerald 2015), whereas local processes such as habitat heterogeneity, species interactions, and ecosystem productivity, tend to exert greater influence on community structure at smaller spatial scales (Brooker *et al.* 2009). Recent studies (Kraft *et al.* 2007; Mouillot *et al.* 2007; Mouchet *et al.* 2013) have demonstrated the functional relationship of co-occurring species could be revealed by environmental constraints over competitive interactions. In turn, examining the organization of coexisting species and the dispersion of their functional traits may help to inform the roles of these processes in structuring ecological communities at different spatial scales.

Three classic assembly rules have been proposed to explain the patterns of species richness and community structure: species interactions, environmental filtering, and neutral processes (Mouchet *et al.* 2013). Species richness and community structure can be influenced by biotic interactions via the principles of limiting similarity

(MacArthur & Levins 1967) and competitive exclusion (Hardin 1960) with the underlying assumption being that species, or their ancestors, have been in competition with one another (i.e. "the ghost of competition past", Connell 1980) and each niche is occupied by the competitively dominant species. Coexistence is promoted by assemblages of species possessing characteristics (i.e. functional traits) that are more dissimilar in relation to one another via complementarity or trait overdispersion. Through a process described as environmental filtering, abiotic factors serve to sort species possessing similar suites of traits adapted to the environmental conditions of a given habitat (Zobel 1997; Weiher *et al.* 1998). This model of environmental filtering due to abiotic factors predicts species coexisting in communities should be more similar to one another in their functional traits than expected by chance. Neutral theory treats species as ecological equivalents, hence their coexistence and persistence is independent of their biological traits (Hubbell 2001). Neutral theory predicts traits possessed by coexisting species would be randomly distributed among species in a community.

Community ecologists have predominately emphasized species identities (i.e., nomenclature) rather than functional traits to understand community organization, even though the utility of functional traits has long been recognized in community ecology (McGill *et al.* 2006). Trait-based approaches to community organization provide the ability for broader applicability and predictability of results (McGill *et al.* 2006; Hulvey & Aigner 2014) as analyses based on the distribution of functional traits can provide insights into the linkages between niche packing and community diversity (Ricklefs & Miles 1994). Functional traits are useful predictors of niche dimensions as the

relationship between morphology and ecology has been well documented for many animal taxa (Miles & Ricklefs 1984; Losos 1990; Winemiller 1991; Hibbitts and Fitzgerald 2005; Silva & Brandão 2010; Montaña *et al.* 2014). Although functional morphology of anurans has been the subject of study at both the larval (Altig & Johnston 1989; Wassersug 1989) and post-metamorphic life stages (Zug 1972; Emerson 1976; Zug 1978; Emerson 1978; Emerson 1985; Emerson 1988), the application of a functional trait framework to understanding how anuran communities are organized has been limited. In their comparison of trait patterns across three biogeographical regions, Ernst *et al.* (2012) concluded local environmental filters were important in in trait composition of regional frog communities. More recently, Schalk *et al.* (2015) demonstrated that environmental filtering played a strong role in the organization of desert frog communities. That study showed a non-random pattern of co-occurring species packed within the morphospace that were more morphologically similar to one another within, than among habitat types (Schalk *et al.* 2015).

Tropical species tend to reveal a greater degree of ecological specialization in their functional traits that are hypothesized to be a product of stronger interspecific interactions (e.g., competition, predation, and mutualisms) (Schemske *et al.* 2009). Neotropical frogs are no exception, as they exhibit high ecomorphological diversity compared to their temperate counterparts (Duellman & Trueb 1994). While some tropical frogs exhibit are dietary specialists (Toft 1980; Parmelee 1999), leaf litter and arboreal anurans trophic generalists show considerable niche overlap in prey taxa (Parmelee 1999), indicative that limiting similarity in not important in driving

assemblage structure. Furthermore, species-habitat associations of local assemblage structure reveal response to environmental gradients rather than patterns consistent with limiting similarity (Watling *et al.* 2005; Dias-Terceiro *et al.* 2005). However, the majority of studies on community organization of post-metamorphic anurans have relied on a taxonomic and habitat framework, rather than examining community organization in the context of functional traits (Wells 2007).

To infer the influence of limiting similarity and abiotic filtering on community organization, I examined patterns of functional trait dispersion among 39 frog species across 12 local species assemblages within the Gran Chaco ecoregion in Bolivia. I examined ecologically relevant functional traits to investigate whether or not coexisting species were more or less similar than predicted at random, and whether species similarity, dispersion, and the total morphospace were correlated with local species richness. I compared observed patterns to null models to assess statistical significance of non-random patterns of community organization. Because previous studies suggest that interspecific interactions are not an important factor driving local assemblage structure of post-metamorphic anurans, I predicted that assemblage trait space would reveal patterns consistent with environmental filtering, and I expected that coexisting species within a given assemblage would possess similar traits.

Materials and Methods

Study site and species

The Gran Chaco, the third largest biome in South America, is a large xeric floodplain that contains vast expanses of semiarid thorn forest and palm savannahs

distributed across the countries of Argentina, Brazil, Bolivia, and Paraguay. The Chaco has a warm, wet summer (November-March) and a cool, dry winter (April-October). In Bolivia, the Chaco occurs in the southeastern portion of the country. Moving west to east, the Chaco transitions from dry Chaco forest to wet Chaco. The dry Chaco is predominately thorn forest, with *Schinopsis lorentzii* and *Aspidosperma quebracho-blanco* being common tree species and cacti (e.g., *Opuntia* sp., *Cleistocactus baumannii*, and *Eriocereus guelichii*) and bromeliads constituting the common understory plants (Navarro & Maldonado 2002). The wet Chaco transitions from dry thorn forests in the east to palm savannahs in the western edge of the Chaco. The lists of coexisting species from the 12 sites used in this study were generated from surveys conducted around the region, which generally ranged from 5 -7 km^2 (Gonzales 1998; Gonzales *et al.* 2006; Montaño & Gonzales 2008; Schalk *et al.* 2013; Schalk unpubl. data) (Figure 1, Appendix A).

Morphological analysis

I measured 13 external morphological traits (Table 1) with dial calipers (precision = 0.1 mm) on specimens deposited in the Museo Noel Kempff Mercado in Santa Cruz de la Sierra, Bolivia (Appendix B). Functional traits were chosen that reflect various facets of anuran trophic ecology (Emerson 1985), habitat use, and modes of locomotion (Emerson 1976; Emerson 1978; Emerson 1985; Emerson 1988). The raw

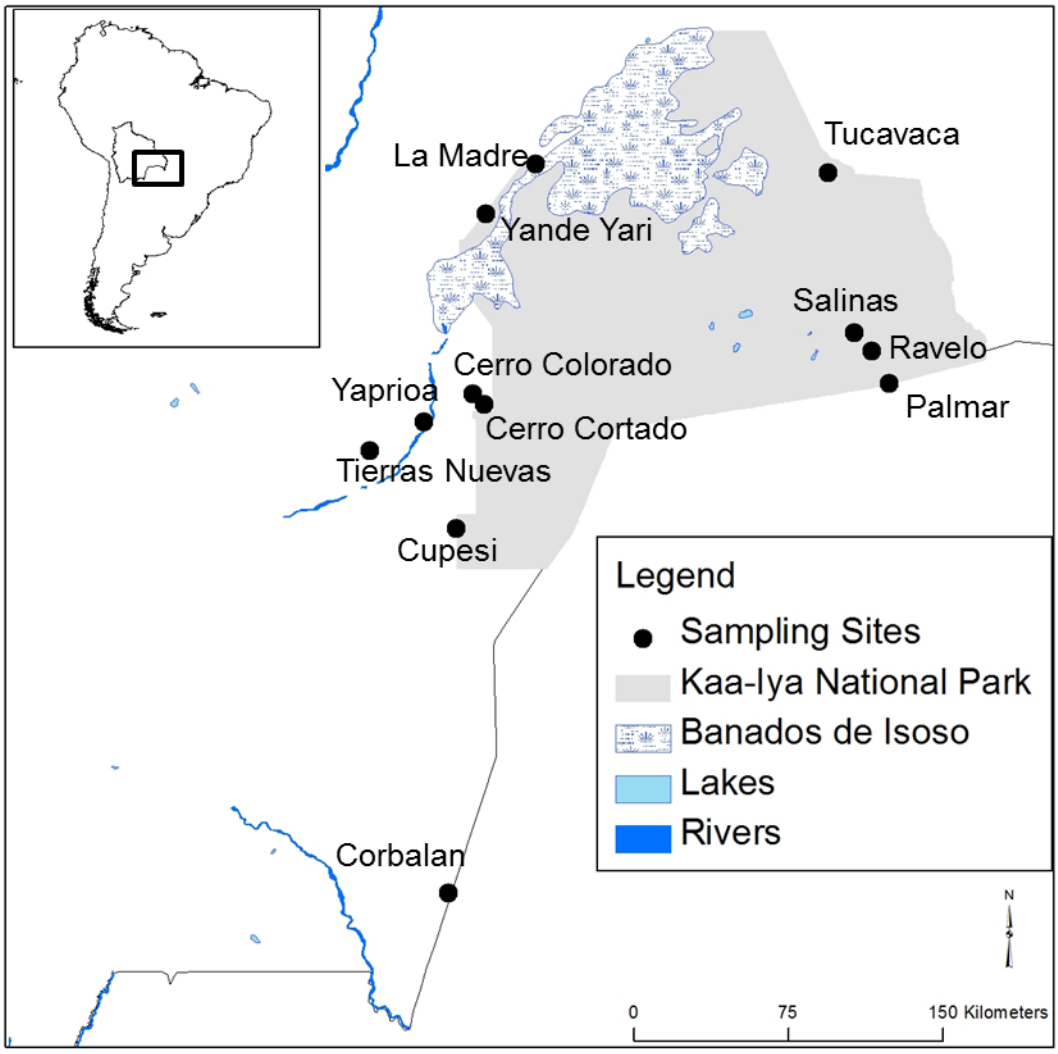

Figure 1. Map of the study region and sampling sites of the Bolivian Gran Chaco.

Table 1. Thirteen functional morphological traits measured on each frog specimen.

Trait	Trait code	Definition of Trait
Snout-to-vent length	SVL	Maximum distance from tip of the snout to behind the vent
Mouth width	MW	Horizontal distance measured outside of mouth at its widest point at the dorsal view
Head length	HL	Horizontal distance from the tip the snout to the posterior margin of tympanum
Head width	HW	Horizontal distance measured passing through the tympanum
Head height	HH	Vertical distance from dorsum to ventrum passing through pupil
Forelimb length	FL	Maximum distance of the ulna/radius to the to the tip of the third digit on the hand
Thigh length	TL	Maximum distance of femur while leg is held in a Z pattern at perpendicular angle to vertebral column
Shank length	SL	Maximum distance of tibiofibula while leg is held in a Z pattern
Tarsus-and-foot length	TFL	Maximum distance from beginning of tarsus to the tip of the third digit on the foot while leg is held in a Z pattern
Inter-orbital width	IW	The shortest distance between upper eye lids
Eye diameter	ED	Horizontal distance from eye margin to eye margin
Inter-narial distance	IN	The distance between the midpoints of naris
Narial-to-mouth distance	ND	Vertical distance from bottom of naris to upper lip of the mouth

data were natural log-transformed before further analyses. To take body size into account, linear regressions between the 12 morphological variables and snout-vent-length (SVL) were performed in JMP Pro.10, and residuals were retained and used as variables in a principal components analysis (PCA). The resulting PC axes described orthogonal, size-free gradients in the morphological space occupied by each species assemblage, which I used to examine among-species differences in functional traits across habitat associations. The PCA was performed in PC-Ord v.6 based on the correlation matrix of all species and traits. Interpretable PCA axes were selected using the broken-stick model as recommended by Jackson (1993).

Analysis of morphological similarity

I recognize there are multiple definitions of the term "assemblage" in the literature; for the scope of this study, I follow the definition proposed by Fauth *et al.* (1996), where they define an assemblage as phylogenetically-related species occurring in the same place at the same time. Morphological dissimilarity within each assemblage and within the pooled regional assemblage was measured as the pairwise Euclidean distances among all species based on the PC axis scores. Sub-matrices containing of each local habitat assemblage were compiled to test for generalizations about species similarity and community organization proposed by Ricklefs and Miles (1994) and Winemiller (1991). I calculated three metrics of morphological dispersion to explore patterns of community organization: First, I calculated the mean nearest neighbor distance (mean-NND), which provides an estimated of ecological similarity among species (Winemiller 1991) and indication of species packing in morphological space.

Second, the standard deviation of NND (SD NND), which provides an estimate of the evenness of species dispersion or packing in morphological space (a lower SD indicates a more regular, or even, dispersion pattern, a pattern consistent with limiting similarity, Ricklefs & Miles 1994). Finally, I calculated the average distance to centroid (CD), which provides an estimate of the total niche space or relative size of the morphological hypervolume defined by each assemblage. Before calculating species morphological similarity, Euclidean distances were weighted to adjust for different amounts of variation modeled by each PC axis (Montaña *et al.* 2014). I performed linear regression analysis to test the relationships between species richness and mean NND, SD NND, as well as for distance to the centroid.

Because phylogenetic relationships among species may influence distribution of traits observed in communities, I tested the null hypothesis of no significant correlation between phylogenetic distances and the size-adjusted morphological data with a Mantel test (Montaña & Winemiller 2013). Besides higher-level topologies, there is no comprehensive phylogeny that includes all 39 species used in this study; therefore phylogenetic distances were measured as the number of nodes separating taxonomic groups from a previously published phylogeny (Pyron & Wiens 2011).

I ran a morphological null model to test the hypothesis that the functional traits are non-randomly distributed across the frog assemblages. The null model was performed in Sampler v.1 (Montaña *et al.* 2014), which generates random species assemblages drawn from the observed species pool, and calculates the nearest-neighbor distances and centroid distances. To generate the random assemblages, species were

chose without replacement in the regional species pool. The null model also assumed all species of the Chaco have equal probability to colonize an association type within this region. Observed values of mean NND, SD of NND, and mean centroid distance (CD) of natural assemblages were compared with values generated randomly for the same number of species as real assemblages. Using the null occurrence matrix and the observed distance matrix, I calculated the randomized statistic (e.g. mean and SD NND, and CD) for each simulated assemblage. Similarly, I compared the mean observed statistics to the random distribution in order to obtain the *p*-value which is the type I error when I reject the null hypothesis (Mouillot *et al.* 2007).

Results

Across the 12 sites, species richness ranged from 8 species to 25 species. Frog species were strongly differentiated by the PCA on the basis of head size and limb length. Principal component axes one and two explained 70.1% of the total variation in species morphology (Figure 2). Principal component axis 1 explained 58.18% of the total morphological variation and described a gradient that reflected morphological differences in skull shape. Species with positive scores on PC1 had relatively wider heads and mouths, longer heads, and deeper heads. For example frogs in the family Ceratophryidae that prey on other vertebrates scored high on PC1 (Schalk *et al.* 2014). Negative values on PC1 were associated with species having narrower, shorter heads and mouths, such as ant and termite dietary specialists in the in the family Microhylidae. Between these two extremes were generalized invertivores (e.g., frogs of the families Hylidae and Leptodactylidae). Principal Component 2 accounted for an additional

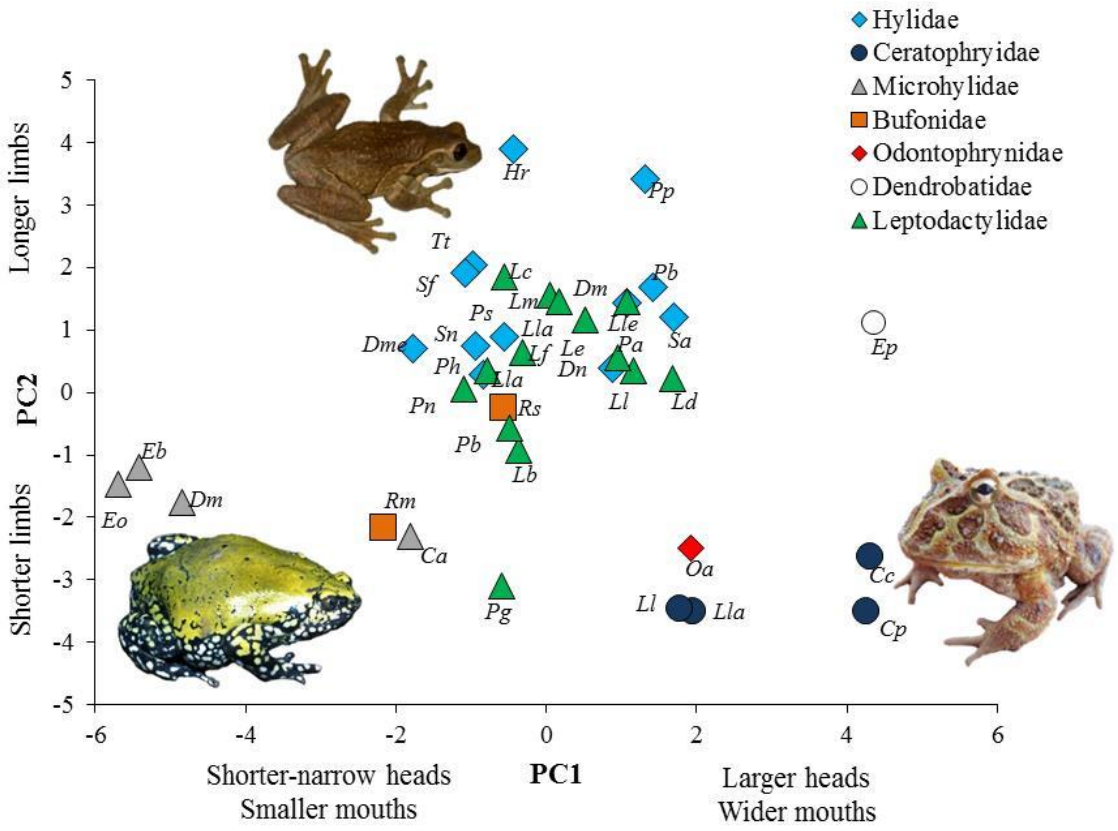

Figure 2. PCA ordination of 39 Chacoan anuran species based on 13 morphological traits. Each point represents the mean value for each species. Points are color-coded by family. PC1 (58.18% morphological variation), PC2 (23.81% morphological variation).

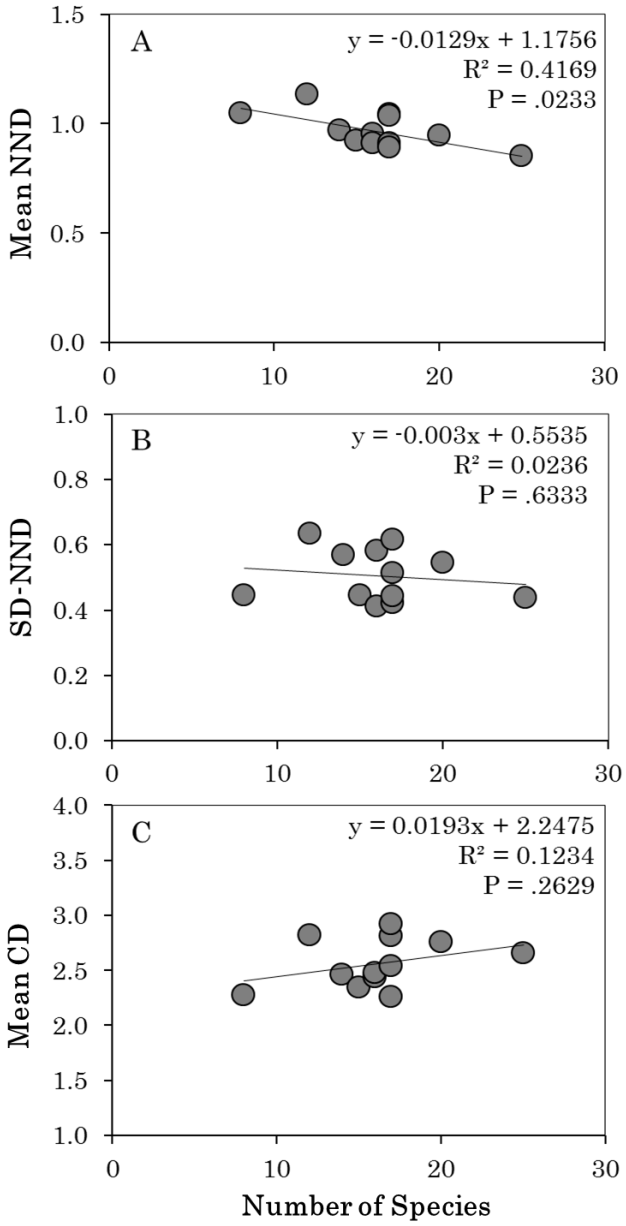

Figure 3. Measures of A) Mean NND, B) SD-NND, and C) Mean CD morphological distances between species in the morphospace plotted as a function of number of species across 12 sites of the Bolivian Chaco. Each point represents the mean value of a single site.

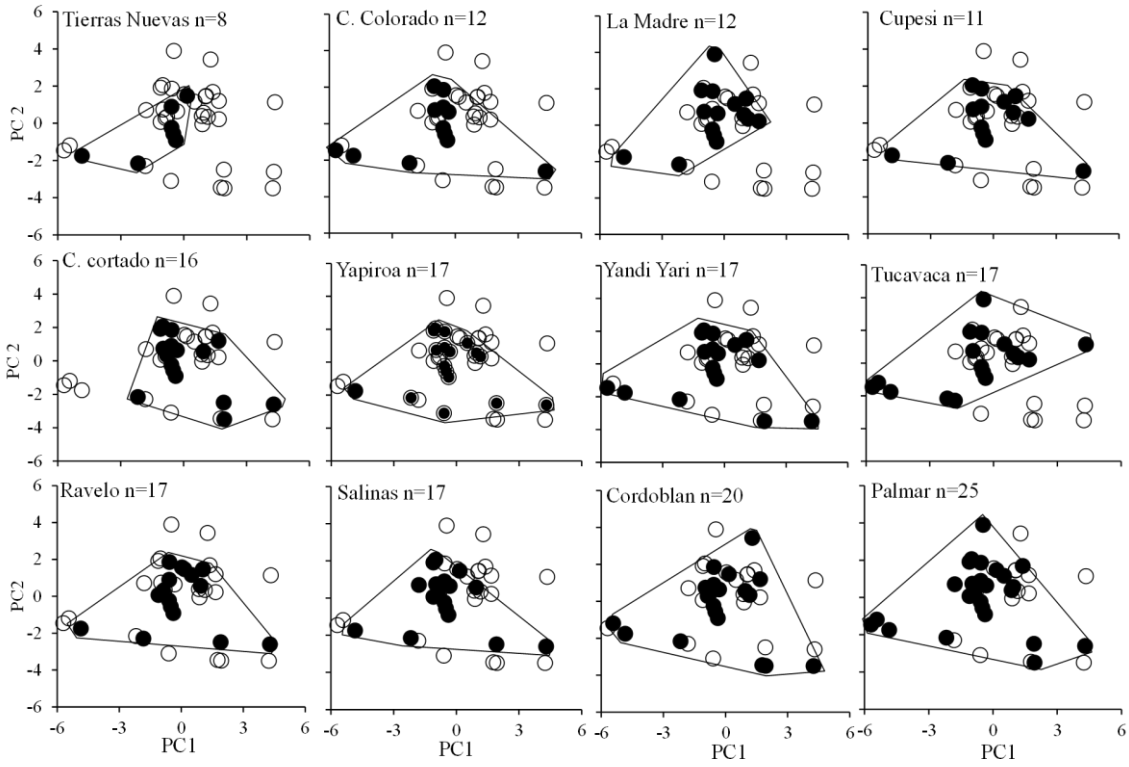

Figure 4. Scatter plots of the PCA1 and PCA2 axes based on size-adjusted 12 morphological traits for 39 frog species of Bolivian Chaco anuran assemblages. Connected lines depict the total morphospace within each site with the total number of species listed on each plot.

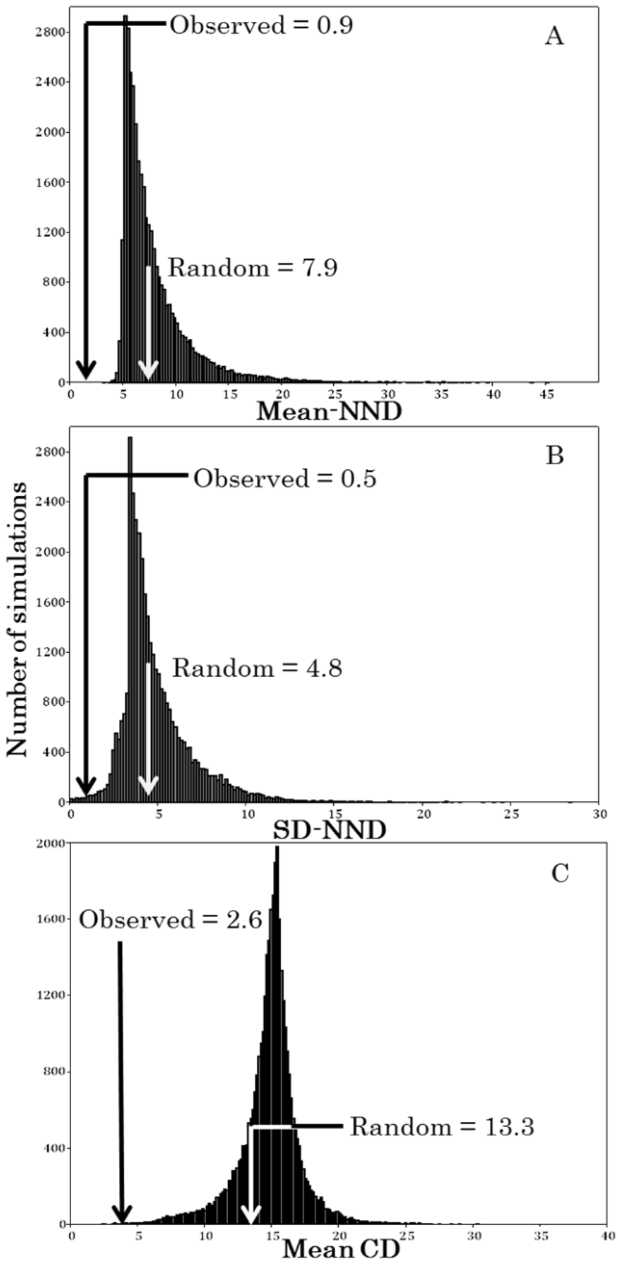

Figure 5. Observed and simulated A) mean and B) standard deviation (SD) nearest neighbor distance (NND), and C) mean centroid distance (CD) among coexisting frog species in the Bolivian Chaco. Black arrows = mean value for observed assemblages, white arrows = mean value for randomly generated assemblages choosing without replacement from the regional species pool using the software SAMPLER v.1 (Montaña et al. 2014).

23.81% of the variation, and described a gradient reflecting morphological differences associated with habitat use. Species with large and positive scores on PC2 had relatively longer hind limbs (arboreal species, e.g., species in the family Hylidae), whereas those species with negative scores had relatively shorter limbs (fossorial species, e.g. Ceratophryids, and Microhylids). The 39 species are represented by a number of families and genera that are not particularly closely related and I did not observe any significant effect of phylogenetic distance on the morphology of these frog species (Mantel test: r = 0.06, $P = 0.12$).

Across the 12 assemblages, mean NND significantly decreased with increasing species richness (Figure 3a, $P = 0.023$) indicating that Chacoan anurans were relatively tightly packed within the morphospace. There was no significant relationship between SD-NND (i.e., community evenness) in relation to species richness (Figure 3b, $P = 0.63$). There was a general trend for assemblages with a higher number of species to possess a larger morphospace (i.e. CD), but this relationship was not statistically significant (Figure 3c, $P = 0.263$). Morphologically distinct species, primarily on the basis of their skull shape, (e.g., Ceratophryids and Microhylids) were added to the periphery of the morphospace, increasing the overall morphological volume (Figure 4), even though it was not statistically significantly larger.

The anuran assemblages from all 12 sites were considered for the randomization analysis. Results from comparisons of the observed data with randomly generated data indicated distribution within morphological space of Chacoan frog assemblages was non-random, as the observed means were all less than the randomly generated mean

values (Figure 5). Taken together, these results suggest support for the hypothesis of environmental filtering over the hypotheses of limiting similarity and neutrality as a driver of the local assemblage structure of the Chacoan anuran community.

Discussion

Species assemblage patterns did not adhere to the assembly rules of neutral theory (Hubbell 2001) and the null model revealed that the assemblages were structured non-randomly with respect to functional trait diversity. Our results suggest that as species were added to the local assemblages, species packing within the morphospace increased. There was no significant relationship or trend between the evenness of species packing and community richness. Finally, our results suggest that niche space in these anuran assemblages does not increase as a function of species richness across gradients of species richness.

Functional traits among species across the 12 assemblages were predominantly associated with trophic ecology and habitat use (head shape and limb lengths). Variation in shape of functional traits is considered important in driving niche partitioning (Brown & Wilson 1956; Hutchinson 1959), and the distribution of functional traits in anuran assemblages should reflect modes of performance and niche diversification (Gomes *et al.* 2009). Limb length is an important predictor of habitat use in anurans (Gomes *et al.* 2009), which in turn allows species to exploit resources (e.g., prey, refuge sites) concomitant with a specific microhabitat. Species using terrestrial and arboreal habitats exhibited similarities in their functional traits (i.e., limb length), a pattern suggesting that environmental factors rather than interspecific interactions certainly might allow the

coexistence of species with similar functional traits in these assemblages. While these patterns are indicative of how assemblages are structured across these sites, coexistence may be facilitated through the specialization and use of specific microhabitats, thereby limiting niche overlap. Conducting this study at smaller spatial scales while collecting data on availability of resources would provide a better insight into the interplay between local resources, functional traits, and interspecific interactions structuring the organization of these local anuran assemblages.

Two feeding guilds are common in tropical leaf litter anurans: ant and termite specialists, and species adapted to eating soft-bodied, mobile invertebrates (Toft 1980). The vertebrate-eating frog guild in the Gran Chaco is unique and contains five species in the Family Ceratophryidae (Scott & Aquino 2005), the highest number of co-occurring vertebrate-eating anurans in the Neotropics. Most of the species within the assemblages in our study were generalized invertivores (possessing intermediate head shape), but both types of trophic specialists (i.e., ant/termite specialists and vertebrate specialists) were also present. Coexisting species in these Chaco assemblages exhibited high overlap in functional traits associated with foraging and feeding, a pattern that is consistent with other studies of post-metamorphic anuran assemblages. The general similarity in functional traits in anuran communities suggests that competition for food resources is not an important driver of post-metamorphic anuran community structure (Toft 1980; Toft 1985; Wells 2007; Schalk *et al.* 2015). I may also infer that limiting similarity is not a strong factor driving community organization across the broader scale of the Chaco ecoregion. However, I assume that the traits I measured were sufficient enough to

capture niche differentiation among co-occurring species. Studies focused on other aspects of ecological performance could identify other mechanisms that also play a role in determining assemblage structure.

Species were significantly more packed within the functional-trait morphospace in relation to increasing species richness, but the overall functional trait-space remained relatively constant through the addition of morphologically distinct species. The closely packed internal spacing was a result of identifiable similarities in functional traits related to foraging and habitat use, suggesting a lack of niche specialization among similar taxa (Winemiller 1991). Alternatively, this pattern suggests that diversification or dispersal limitation may play a larger role at broad spatial scales (Ricklefs 2012). The terrestrial (predominately frogs in the family Leptodactylidae) and arboreal (family Hylidae) guilds exhibited similarities in mouth shape associated with a predominately invertivorous diet. Within each respective guild, species overlapped considerably in limb shape, which is primarily associated with habitat use (Gomes *et al.* 2009). The morphologically distinct species at the periphery of community morphospace exhibited trait differences that were primarily associated with specialized foraging ecologies, such as ant and termite eating specialists with their extremely narrow mouths (family Microhylidae), and those species with extremely large mouths capable of consuming large-bodied vertebrates (family Ceratophryidae; Schalk *et al.* 2014; Schalk & Fitzgerald 2015). These species were also characterized by relatively shorter limbs, reflecting their fossorial lifestyle (Emerson 1976; Schalk *et al.* 2015). Similar patterns of trait-community assembly have been observed for communities of bats, (Findley & Black 1983), fishes (Winemiller 1991),

birds (Ricklefs & Travis 1980; Ricklefs 2012), and desert frogs (Schalk *et al.* 2015) where species tended to join the periphery of the morphospace while overall community morphospace remained constant. The congruence in morphological patterns across widely different taxon groups and regions is likely result of similar underlying linkages between functional traits and the broad spatial scale at which these studies were conducted.

Our results provide insights into the role of environmental filtering in structuring local anuran assemblages in accordance with their functional traits. To further understand processes governing community membership in neotropical frog communities, studies at even smaller scales may provide insights of whether other assembly rules (e.g., limiting similarity) might support coexistence (Montaña et al. 2014). Studies have highlighted the importance of how processes of community organization can vary with spatial scale (Weiher & Keddy 1995; Dias-Terceiro *et al.* 2015; Ryberg & Fitzgerald 2015). Because a number of independently studied amphibian communities tended to exhibit similar patterns of overlapping functional traits, I identified environmental filtering of abiotic factors as an important driver of community assembly of post-metamorphic anurans at smaller spatial scales. For example, in a species-rich stream system in Madagascar, coexisting tropical tadpoles exhibited high functional redundancy, suggesting a response to environmental filters in the stream habitats (Strauß *et al.* 2010). At sites in lowland rainforests in Africa, patterns of assembly in leaf litter anurans in the lowland rainforests appeared to be the product of species associations to environmental gradients. In that system, interspecific interactions

did not play a large role in community organization (Ernst & Rödel 2006). Although not studied in the context of functional trait diversity, Watling (2005) found that leaf litter anurans exhibited local variation in community structure, which he attributed to edaphic gradients across sites.

I have presented insights into the processes structuring local anuran communities, in terms of how functional trait space may be expanded, and how species are packed into morphospace as they are added to communities. I can conclude that anuran community structure was not random or neutral, and across many local assemblages species packing occurred due to co-occurrence of species with similar functional traits related to diet and habitat affinity. Overall functional trait space in the communities was broadened by inclusion of trophic specialists with correspondingly extreme morphological traits.

Several implications for conservation are apparent from our perspective on anuran community assembly based on functional traits. Over one third of the amphibian species are experiencing population declines (Stuart *et al.* 2004), and these species occur in structured communities. A functional trait framework such as I provide here allows for general predictions about the consequences of landscape changes for ecological communities in threatened habitats. Ernst *et al.* (2006) observed a loss in functional diversity of tropical anurans in response to timber harvesting. Leavitt and Fitzgerald (2013) demonstrated that habitat fragmentation causes community disassembly, with ecological specialists being the first species lost from the local assemblages. Understanding the role of functional traits as they relate to community structure can provide important insights for community-level conservation and restoration (Hulvey &

Aigner 2014). The Gran Chaco ecoregion is experiencing some of the highest rates of deforestation globally (Zak *et al.* 2004; Frate *et al.* 2015). As landscapes continue to be altered in the Gran Chaco, communities may be disassembled and re-assembled in accordance with the performance of species functional traits in the altered environmental gradients.

CHAPTER III

TROPHIC PLASTICITY, ENVIRONMENTAL GRADIENTS, AND FOOD WEB STRUCTURE OF TROPICAL POND COMMUNITIES

Introduction

A fundamental goal in ecology is to determine the mechanisms responsible for community organization and species coexistence (Chesson, 2000; Siepielski & McPeek, 2010). Studies of community structure can generate hypotheses about how and why attributes of communities allow their assembly and persistence within certain environments. Food webs are depictions of consumer-resource interactions and can provide insights as to trophic relationships and resource use amongst coexisting species.

Freshwater pond communities exhibit strong patterns in species composition in response to environmental gradients such as ecosystem size, disturbance, and productivity, serving as excellent systems for studies of food web structure (De Meester *et al.* 2005, Werner *et al.* 2007; Wellborn *et al.* 1996; Williams 2006). Most studies of species inhabiting lentic water bodies have attributed patterns of community structure as a response to pond permanence, and tradeoffs that are associated with the constraints of pond drying and predation (Wellborn *et al.* 1996). In addition to pond hydroperiod, canopy cover has also been shown to affect structure of pond communities (Werner & Glennemeier 1999, Pazin *et al.* 2006, Werner *et al.* 2007, Binckley & Resetarits 2009). Werner *et al.* (2007) documented a decline in species richness in ponds with longer hydroperiods, which was clearly attributed to the presence of fish in more permanent

ponds. Patterns of amphibian distribution among fishless ponds were strongly linked with abiotic variables, specifically canopy cover and pond area (Werner *et al.* 2007). Some species appeared to be intolerant to increased canopy cover, and changes in their distribution were associated with increasing forest encroachment around breeding ponds (Skelly *et al.* 1999, Werner & Glennemeier 1999). Canopy cover affects the resource type and quality by reducing pond productivity and this in turn affects consumer developmental rates (Werner & Glennemeier 1999, Skelly *et al.* 2002, Halverson *et al.* 2003).

Previous studies have converged on the pattern that less-predictable environments (i.e., ponds with a shorter hydroperiod) tend to support food webs with shorter food chains. However, all of these studies have been conducted in temperate regions. There is much to learn from examination of the structure of tropical pond communities and how community structure of tropical species may or may not follow the patterns and trade-offs that are apparent in temperate systems. Tropical species, in general, tend to exhibit a greater degree of ecological specialization in their functional traits (Schemske *et al.* 2009). Consumers in temporary tropical ponds, such as tadpoles, exhibit high eco-morphological diversity compared to their temperate counterparts (Altig & McDiarmid, 1999; Altig & Johnson, 1989). Thus, different patterns may emerge when comparing food web structure in response to environment gradients in the tropics.

I sought to examine variation in food web structure of tropical ponds across environmental gradients by using ^{13}C and ^{15}N stable isotopes to gain insight into the structure of food webs along gradients of habitat disturbance (pond drying), ecosystem

size (pond area), and productivity (canopy cover). I predicted larger and more stable habitats would support food webs that exhibit higher trophic redundancy and greater trophic diversity than more ephemeral habitats. I predicted that that less productive (more shaded) habitats would exhibit lower trophic redundancy and diversity because fewer species would be tolerant to increased canopy cover. I further predicted that the greater ecomorphological diversity of consumers within these tropical ponds would result in increased specialization of their trophic ecology as compared to their temperate counterparts.

Materials and Methods

Study site

This study was conducted in the semi-arid thorn forests of the Gran Chaco ecoregion of south-eastern Bolivia. The region has a warm, rainy season (November–March) and a cool, dry season (April–October). The surrounding vegetation in this region is predominately thorn forest; common tree species include *Schinopsis lorentzii* and *Aspidosperma quebracho-blanco* with cacti (e.g. *Opuntia* spp., *Cleistocactus baumannii* and *Eriocereus guelichii*) and bromeliads constituting the common understory plants (Navarro & Maldonado 2002). The study area is located in one the most xeric regions of the Bolivian Chaco with annual rainfall and temperature averaging 513 mm and 24.6°C, respectively (Navarro & Maldonado 2002).

Surveys and sample preparation

I surveyed 13 ponds at two sites, Kuaridenda (19.17°S, 62.53°W; N = 5 ponds) and Yaprioa (19.61°S, 62.57°W; N = 8 ponds) in the indigenous territory of Isoso,

Cordillera Province, Santa Cruz Department, Bolivia. Ponds were both natural and anthropogenic and arrayed along natural gradients of disturbance regime, ecosystem size, and canopy cover. Reproduction is concentrated within the rainy season for the taxa that use these ponds for breeding (Schalk & Saenz, 2016; Schalk *et al.* 2014). All the anurans in the study area are pond breeders and have exotrophic tadpoles (Perotti, 1997).

I collected tadpoles, macroinvertebrates, and fish at the beginning (December 2010/January 2011) and at the end (April 2011) of the rainy season using an area-based sampler and dipnet surveys. I used a 120-L plastic trashcan to create an area-based sampler (height = 0.43 m, sampling area = 1.3 m^2). I placed the sampler every 3 m along the pond's longest axis and cleared the sampler using a dip net (mesh size = 2 mm). To ensure that all organisms had been removed from the sampler, I conducted at least ten sweeps with the dip net and ten additional sweeps after the last animal was collected (Werner *et al.* 2007). During each survey period, I retained samples of tadpoles, fishes and aquatic invertebrates per pond for isotopic analyses.

Tissue samples for stable isotope analysis were taken from three specimens of each species during each survey period. In addition I collected potential basal sources (sediment, seston, and C3 plants) from each pond by hand. I collected seston by filtering 100 ml of water through pre-combusted Whatman GF/F filter. Samples for stable isotope analysis were preserved in salt as described by Arrington & Winemiller (2002). In the laboratory, tissues were soaked in distilled water for 4 h, then rinsed again to remove salt. Samples were then dried at 60°C for 48 h in a drying oven. Dried samples were ground to a fine powder with a mortar and pestle, and then stored in clean glass vials.

Subsamples of each ground sampled were weighed (1.5-3 mg) and packaged into a Ultra-Pure tin capsules (Costech Analytical, Valencia, California, USA) and sent to the Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, for analysis of stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). During mass spectrometry, two different standards were processed between every 12 samples, and precision was ±0.22 ‰ for $\delta^{13}C$ and ±0.20 ‰ for $\delta^{15}N$. Isotope ratios were reported in parts per thousand (‰) standardized in relation to reference material (Pee Dee Belemnite for C, atmospheric nitrogen for N) and reported as $\delta X = [(R_{sample}/R_{standard})-1)]*10^3$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$

I quantified environmental gradients at each pond monthly from January - April 2011. Canopy cover was estimated using a spherical densitometer with a measurement taken at every 5 m along the pond's longest axis. At each sampling site, four readings were taken, one facing each cardinal direction. Canopy cover was estimated as the average of four readings taken at each measurement site, then averaging those to get an overall mean canopy cover for a pond (Werner *et al.* 2007). Pond area was determined by using a measuring tape to measure the pond's longest and widest axis and using the formula of an ellipse to calculate surface area. Hydroperiod (the number of days the ponds held water within the study period) was determined by checking the ponds for water approximately every two weeks. When a pond was found to be dry, I assumed the pond dried in the midway point between surveys.

Data analyses

Trophic position from isotopic data (TP SIA) was estimated based on fractionation of ^{15}N between the consumer and basal production sources collected from its locality (Vander Zanden & Rasmussen, 1999; Post, 2002) using the formula:

$$\text{Trophic position} = [(\delta^{15}N_{consumer} - \delta^{15}N_{reference})/2.8] + 1$$

where $\delta^{15}N_{reference}$ was the mean $\delta^{15}N$ of basal sources (C3 plants, seston, sediment), and 2.8‰ is the mean trophic fractionation (Vanderklift & Ponsard, 2003).

To examine food web structure, I used community-wide metrics for food webs (Layman et al., 2007). I examined the degree of trophic redundancy (mean nearest-neighbor distance [NND]), evenness of trophic niches (standard deviation of NND [SD-NND]), average degree of trophic diversity (centroid distance [CD]), the magnitude of food web trophic diversity (convex hull of the total area [TA]), the vertical structure within the food web ($\delta^{15}N$ range [NR]), range of basal resource diversity ($\delta^{13}C$ range [CR]) (Layman *et al.* 2007). It is known that these metrics may be biased when isotopic signatures of basal sources are not considered, because $\delta^{13}C$ values might be influenced by physicochemical and other environmental characteristics across systems (Hoeinghaus and Zeug, 2008). In this study however, these metrics are appropriate given that basal sources did not vary across ponds within a survey period (Figure 6 and Appendix C). The SIAR (Stable Isotope Analysis in R) package in the R software version 3.0.2 (R Core Team, 2013) was used to calculate food web metrics. I used generalized linear models (GLMs; using a normal distribution and link function) to explore the relationships between food web structure and environmental gradients. Because there

was no relationship between pond area, hydroperiod, or canopy cover, we analyzed the influence of these gradients on food web structure separately. Data were log transformed prior to analyses that were conducted using the statistical software PAST (Hammer *et al.* 2001).

Results

Samples from 331 consumers (early season = 158, late season = 172) and 98 basal sources (early season = 54, late season = 44) were analyzed for stable isotope ratios. Consumer samples included 11 tadpole species, 4 fish species, and 16 macroinvertebrate taxa. Consumers $\delta^{13}C$ ranged from –35.2‰ to –16.8‰ at the beginning of the rainy season, and from –33.8‰ to –17.9‰ at the end of rainy season. Consumer $\delta^{15}N$ ranged from 4.2‰ to 15.9‰ at the beginning of the rainy season, and from 4.2‰ to 19.8‰ at the end of rainy season. Producer $\delta^{13}C$ ranged from –31.3‰ to –19.4‰ at the beginning of the rainy season, and from –31.3‰ to –20.5‰ at the end of the rainy season. Producer $\delta^{15}N$ ranged from 4.5‰ to 13.4‰ at the beginning of the rainy season, and from 4.2‰ to 13.7‰ at the end of rainy season.

Trophic positions

The stable isotope bi-plots indicated consumers became more enriched in $\delta^{15}N$ at the end of the rainy season (Figure 6). Most consumer $\delta^{15}N$ values were consistently higher than those of basal production sources, but there were some exceptions. The $\delta^{15}N$ ratios of seston were more variable and at times, more enriched than consumers.

Macroinvertebrates and tadpoles revealed high degrees of isotopic variation across space and time, and estimates of trophic position for several taxa ranged by more

than one level (Figure 7). Consumers tended to occupy higher trophic positions at the end of the rainy season (Figure 8). At the beginning of the rainy season, mean trophic position of consumer taxa ranged from 0.14 (*Leptodactylus bufonius* tadpoles) to 3.9 (*Ceratophrys cranwelli* tadpoles). At the end of the rainy season, mean trophic position of consumer taxa ranged from 0.25 (Hydrophilidae beetles) to 5.3 (*Scinax nasicus* tadpoles) (Fig. 2). During the early rainy season, most taxa tended to have lower trophic positions compared to other seasons (tadpole mean ± SD = 1.4 ± 0.9, macroinvertebrate mean ± SD = 1.5 ± 0.7, fish mean ± SD = 1.6 ± 0.6; Fig. 3a). The few taxa that had higher trophic positions were predominately macroinvertebrates (Figure 8a). At the end of the rainy season, tadpoles had higher mean trophic positions and distributions that tended to be left skewed compared to other taxa in the early rainy season (tadpole mean ± SD = 3.1 ± 1.3, macroinvertebrate mean ± SD = 1.7 ± 1.1, fish mean ± SD = 2.1 ± 0.5; Figure 8b). Macroinvertebrates did not occupy highest trophic positions during the late rainy season (Figure 8b).

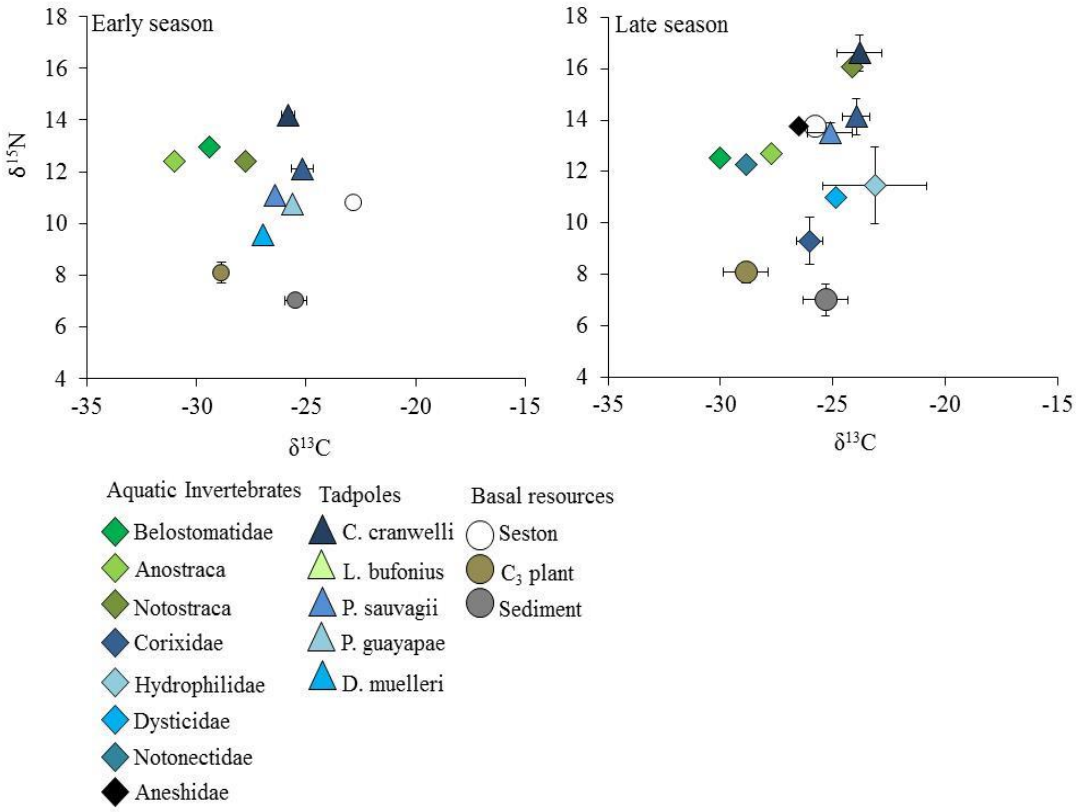

Figure 6. Biplots of ^{13}C and ^{15}N for tadpoles, aquatic invertebrates, and three basal sources for one of the ponds (P-6) in the study area. Isotope ratios were compared for certain taxa that occurred near the beginning (Early season) and end (Late season) of the annual rainy season. Tadpoles include: Cc: *C. cranwelli*, Lb: *L.bufonius*, Ps: *P. sauvagii*, Dm: *D. muelleri*, Pg: *P. guayapae*.

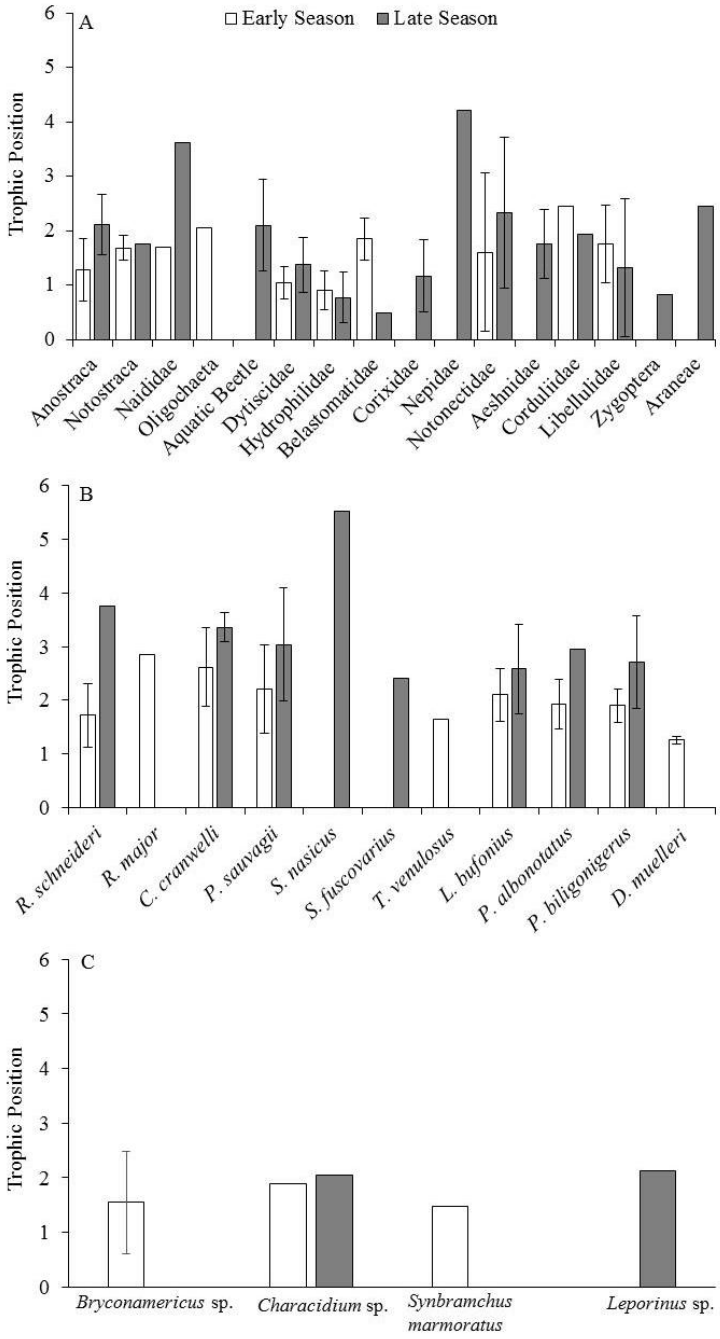

Figure 7. Mean trophic position (+1 standard deviation) of A) macroinvertebrates, B) tadpoles, and C) fish collected in 13 ponds at the beginning (white bars) and end (gray bars) of the annual rainy season.

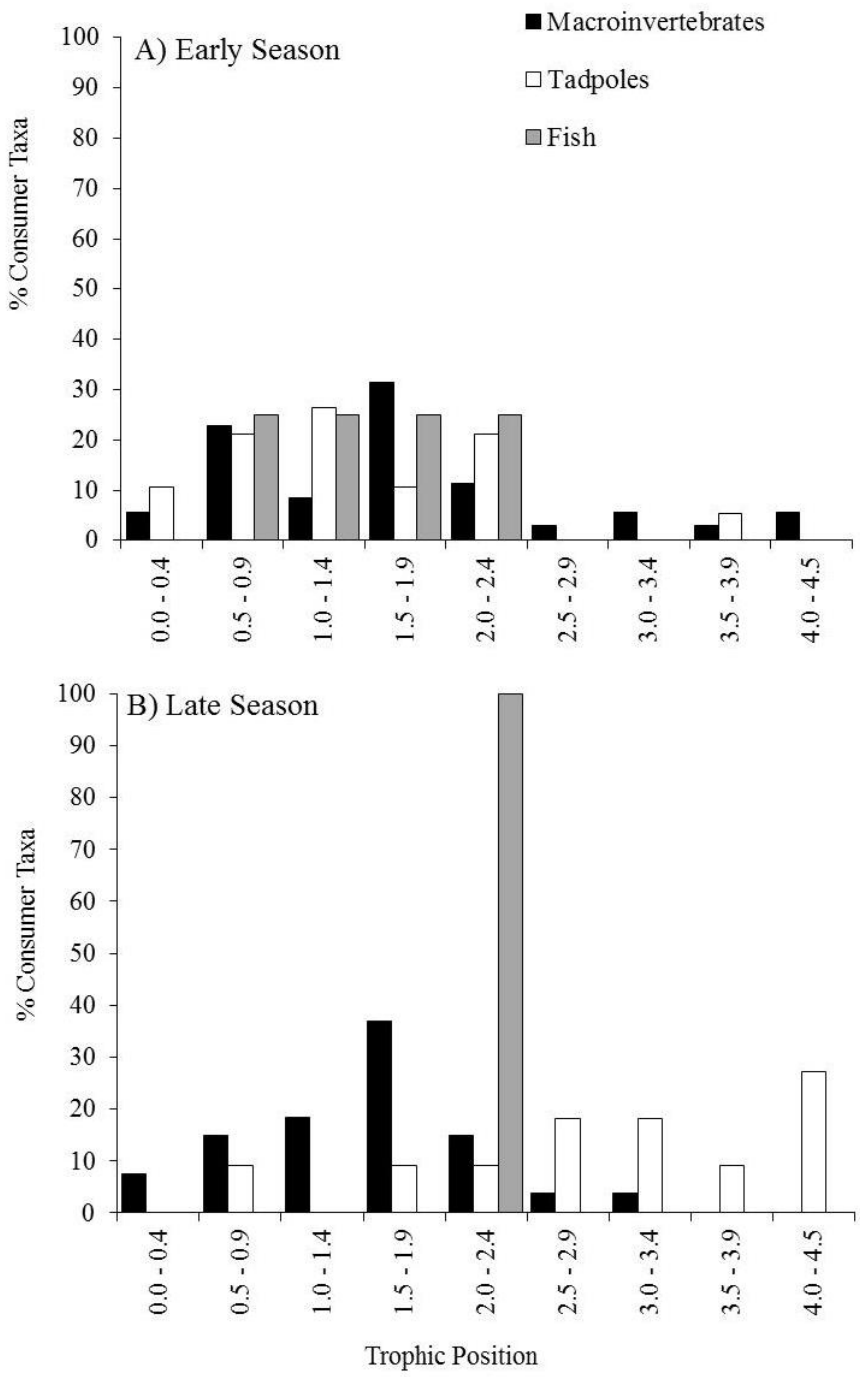

Figure 8. Frequency histograms of trophic positions of macroinvertebrates (black bars), tadpoles (white bars), and fish (gray bars) collected across 13 ponds at the A) beginning and B) end of the annual rainy season.

Food web structure

Food web structure varied along environmental gradients across seasons. At the beginning of the rainy season, consumers were significantly more packed within the trophic niche space with increasing pond size (GLM, $R^2 = 0.17$, $p = 0.02$; Mean NND = 0.40285 - 0.16142log 10 [area]) (Figure 9). Trophic diversity was significantly lower with increasing canopy cover at the beginning of the rainy season (GLM, $R^2 = 0.47$, $p < 0.001$; CD = 0.68649 -0.16587log 10 [canopy cover]) (Figure 9). At the end of the rainy season, more stable environments (i.e., longer hydroperiod) had significantly greater trophic diversity (GLM, $R^2 = 0.29$, $p = 0.047$; CD = -1.532 + 1.088log 10 [hydroperiod]) as well as had a greater range of basal resource diversity as indicated by a greater range of $\delta^{13}C$ (GLM, $R^2 = 0.0073$, $p = 0.01$; Range $\delta^{13}C$ = 1.741 - 2.3367log 10 [hydroperiod] (Figure 9). Trophic niches became less even in their distribution with increasing canopy cover at the end of rainy season (GLM, $R^2 = 0.19$, $p = 0.036$; CD = 0.13948 - 0.18091log 10 [canopy cover]) (Figure 9). Neither the range of trophic diversity (Range N) nor the extent of trophic diversity (TA) exhibited any structure along any of the three environmental gradients (Figure 9).

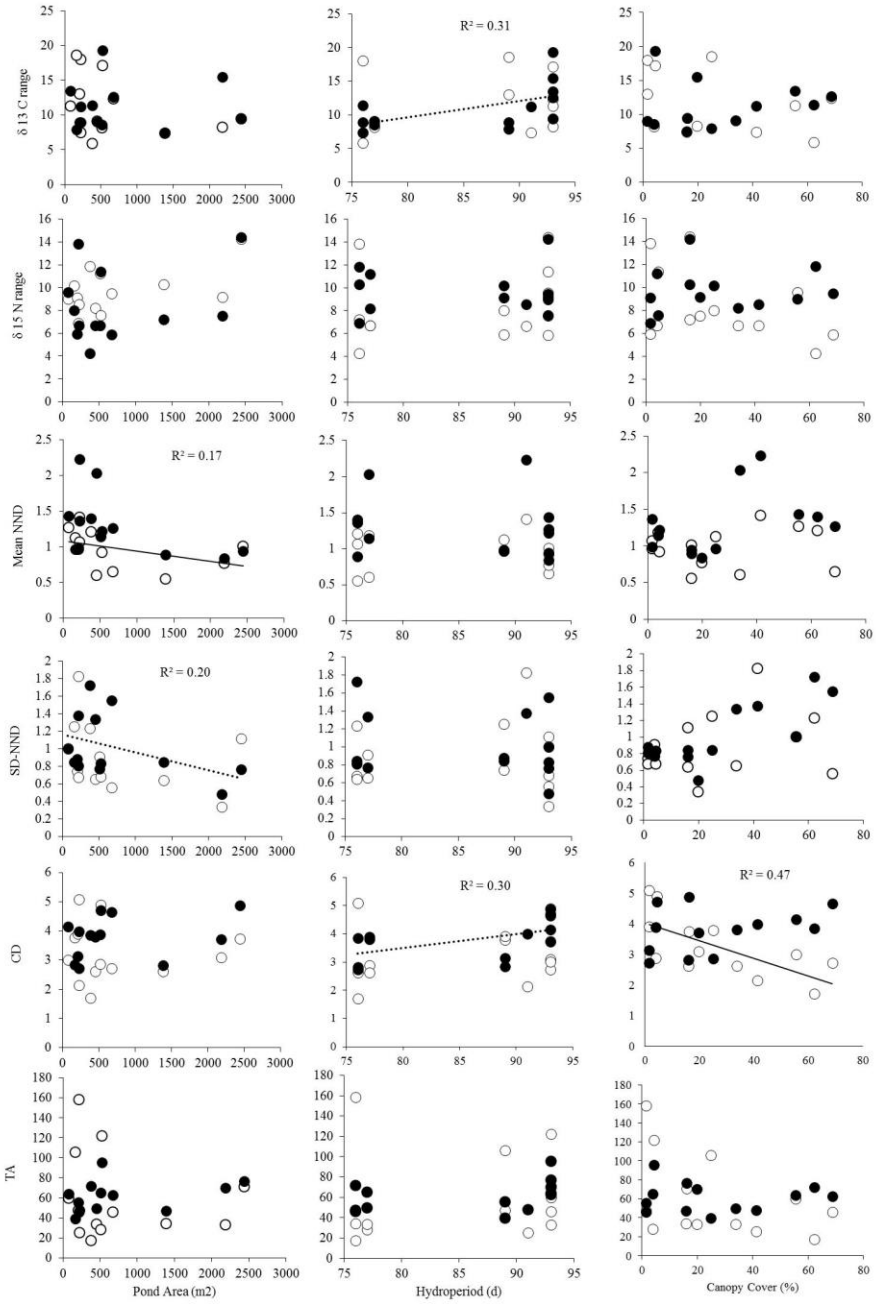

Figure 9. Metrics of food web structure in relation to the environmental gradients defined by pond area (first column), hydroperiod (second column), and canopy cover (third column) at the beginning (white circles) and end (black circles) of the annual rainy season. Significant relationships between food web structure and an environmental gradient are depicted by the presence of a solid line (early season) and dashed line (late season).

Discussion

The macroinvertebrate and tadpole consumers within these ponds food webs exhibited considerable range in their trophic level across both space and time. On average, many of the tadpoles can be classified as omnivorous, but the variation in trophic level ranged from herbivorous to carnivorous, even within a single species. Tadpoles were generally thought of as being herbivorous or detritivorous (Altig *et al.* 2007). In contrast, these results demonstrated that tadpole consumers were feeding at a much higher trophic level than previously thought, corroborating results from previous studies (Petrenka & Kennedy, 1999; Schiesari *et al.* 2009). While tropical species tend to possess greater degree of ecological specialization in their functional traits (Schemske *et al*. 2009; Altig & Johnson, 1989), these results suggest that increased morphological specialization does not necessarily translate into trophic specialization for many of the tadpole and macroinvertebrates in these ponds. Indeed, given that the presence of the ponds is extremely dynamic in the tropical chacoan landscape; opportunistic consumers capable of consuming a broad range of resources when they enter a pond would probably have an ecological advantage.

Experiments have demonstrated that larval amphibians are very labile in their trophic ecology. Diet in tadpoles is context-dependent and often mediated by the density of conspecifics, heterospecific competitors, or non-consumptive effects of predators (Caut *et al.* 2013, Arribas *et al.* 2015, Greig *et al.* 2013). In a temperate amphibian community, the presence of a crayfish competitor, for example, forced tadpoles to shift to a more detritus-based diet and consume resources that were depleted in $\delta^{13}C$ (Arribas

et al. 2015). Trade-offs between foraging activity and predation risk are well-established in tadpole communities (Werner and& Anholt, 1993, Dayton &and Fitzgerald, 2001), but whether this trade-off drives variation in trophic position of the macroinvertebrate and tadpole consumers within these tropical ponds needs to be tested through additional experiments (*sensu* Caut *et al.* 2013, Arribas *et al.* 2015). A line of experimental research that manipulates the density or presence of predators or competitors would be particularly fruitful for identifying the mechanisms that mediate the variation in trophic ecology of consumers in tropical pond communities. While I echo the call from Altig *et al* (2007) to understand the functional role of these tadpole consumers within these food webs, it is important to recognize that even though the isotopic values of certain species suggested they were feeding on animal material, the functional role of these species in the communities remains unclear. Whether these tadpoles were influencing community structure as predators (Schalk *et al*. 2014) or whether they were scavengers on animal material needs further exploration.

Certain aspects of food web structure were nonrandom in relation to certain environmental gradients associated with pond habitats. The range of $\delta^{13}C$ significantly increased with increasing hydroperiod at the end of the late season. In ponds with longer hydroperiods, a greater range of basal sources such as seston or input from leaf fall may establish (Schreiver, 2015). Variability in hydroperiod can also influence food web structure and function (Schreiver & Williams, 2013; Schreiver 2015). In temperate pond communities, Schreiver and Williams (2013) found food chain length was positively correlated with more stable (i.e. longer hydroperiod) environments. Interestingly, in

contrast to the well-established pattern in temperate areas, I found no relationship between the vertical structure within the food webs (range of $\delta^{15}N$) and any of the environmental gradients I measured. When comparing types of ponds it is important to differentiate between temporary ponds and the variation ponds exhibit during drying (i.e., ephemeral ponds; Perotti *et al.* 2011). Temporary ponds from temperate regions dry in a more predictable annual fashion compared to their tropical counterparts (Perotti *et al.* 2011; Schreiver & Williams, 2013; Schreiver 2015). Ponds in the Chaco where I carried out this study were much more dynamic than shown in many studies of temperate ponds and may dry and refill multiple times within a single rainy season (Schalk 2016; Schalk & Saenz, 2016). The impact of this unpredictable variation in pond drying is reflected by the pervasiveness of many complex oviposition strategies of frog species in the region. In particular, many species lay their eggs in foam nests or terrestrial nest chambers (Crump, 1974; Duellman & Trueb, 1994; Perotti 1997; Schalk & Saenz, 2016). One prevailing hypothesis is that these complex oviposition strategies are believed to have evolved as a means to protect eggs from desiccation associated with unpredictable pond drying (Duellman & Trueb, 1994; Crump 1974, 2015). Moreover, species that use ephemeral ponds in the tropics seem to exhibit a greater degree of trophic plasticity, which may allow them to utilize whatever resources are available in the pond regardless of when it is colonized. Thus, because these consumers use an opportunistic feeding strategy, it stands to reason that there was no relationship between food web vertical structure and any of the environmental gradients.

Trophic diversity decreased with increasing canopy cover at the beginning of the rainy season. Canopy cover can be an important resource gradient for consumers within ponds (Schiesari, 2006), but at least in temperate areas, it is also important in influencing the distribution of freshwater species across ponds (Skelly *et al.* 2002, 2014; Schiesari 2006, Werner & Glennemeier 1999). Some species in this study that occurred in ponds at the beginning of the rainy season may have been intolerant to increased shading, thereby constraining their distribution from canopy-shaded ponds, which resulted in the decreased trophic diversity I observed. Alternatively, because the ponds were newly formed at the beginning of the rainy season, the shaded ponds simply may not have been colonized by some of the species. Evidence for the delayed colonization of some of the canopy-shaded breeding ponds was that at the end of the rainy season, trophic diversity increased with increasing hydroperiod. Intermediate hydroperiod ponds that dry in a predictable fashion tend to harbor the greatest diversity of amphibian and macroinvertebrate species richness and diversity (Semlitsch *et al.* 2015, Werner *et al.* 2007, Schreiver & Williams, 2013). Ponds with longer hydroperiods may have been more saturated with consumers as the breeding season progressed. The colonization of these habitats by species with broad trophic niches naturally lead to an increase in overall trophic diversity by the end of the season.

Overall, I may conclude that in this tropical ephemeral pond system changes in food web structure among ponds was mostly driven by pond size and the distribution of trophic guilds. At the beginning of the rainy season, there was evidence of increasing trophic redundancy (i.e., consumers were more packed within the trophic space) in

relation to increasing pond size. Habitat area is recognized as a primary factor controlling species composition and abundance, and subsequently, food web structure (Montaña *et al.* 2015, Semlitsch *et al.* 2015, McHug *et al.* 2015). Larger habitats are more easily colonized by immigrants, resulting in a positive species-area relationship (Montaña *et al.* 2015) that will enhance functional diversity and allowing for more coexistence of species via partitioning of abundant food resources. Also, early in the rainy season, the diversity of basal resources may have not had the opportunity to establish as many of the ponds were recently formed. Reduced resource diversity may have resulted in increasing trophic redundancy as many of the consumers were utilizing similar resources. I also observed that trophic niches were more even with increasing pond size at the end of the rainy season. This could be due to the fact that towards the end of the rainy season the ponds were drying and resources were potentially becoming limited.

Because of the ephemeral nature of these ponds in this region, many of the taxa using these ponds serve as a link between the aquatic and terrestrial environments (Whiles *et al.* 2006). Tadpoles are important consumers in these aquatic environments as they affect resource availability and quality (Whiles *et al.* 2006, 2010). Anuran species are also threatened with population declines, which are especially acute in the tropics (Young *et al.* 2001, Lips *et al.* 2006, Altig *et al.* 2007). In tropical stream systems, the loss of larval amphibian consumers due to chytrid fungus had substantial effects on ecosystem structure and function (Whiles *et al.* 2006, 2010). Because the consumers in the Chaco ponds, including tadpoles, exhibit a high degree of trophic plasticity and are

able to utilize a broad range of resources within a pond, loss of these consumers may have substantial effects on ecosystem processes within ponds, which also may have cascading effect on ecosystem processes in both the aquatic and terrestrial environments.

CHAPTER IV

ENVIRONMENTAL DRIVERS OF ANURAN CALLING PHENOLOGY IN A SEASONAL NEOTROPICAL ECOSYSTEM∗

Introduction

A central goal in ecology is to understand the factors that drive the variation in species phenologies, as they can provide insights to interactions and coexistence amongst species (Schoener 1974). An organism's phenology, i.e. the occurrence of vital cyclic activities within the year, is the product of abiotic and/or biotic factors, such as avoidance of predators or competitors, or tracking certain seasonal resources such as water, light or nutrients (Van Schaik *et al*. 1993; Bradshaw & Holzapfel 2007). However, the relative impact of these abiotic and biotic factors on an organism's phenology varies and is dependent on the appropriate timeframe (i.e. short vs. long-term). Activity cycles of ectothermic animals, such as amphibians, are strongly influenced by abiotic factors, principally temperature and rainfall, due to their permeable skin and aquatic reproduction (Duellman & Trueb 1994; Hartel *et al*. 2007). The timing of these events can have important consequences for the timing of species interactions, and for those species with complex life cycles (e.g. amphibians), this can carry over to

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affect interactions occurring across multiple life stages and influence community composition at local scales (Parmesan 2007; Yang & Rudolf 2010; Todd *et al.* 2011).

Anuran communities exhibit latitudinal variation in the abiotic factors that drive reproductive activity, and within an assemblage, exhibit species-specific responses to these abiotic factors both in short-term (daily) and long-term (seasonal) periods (Aichinger 1987; Moreira & Barreto 1997; Oseen & Wassersug 2002; Kopp & Eterovick 2006; Saenz *et al*. 2006; Both *et al*. 2008; Canavero *et al*. 2009; Narins & Meenderink 2014). In their examination of geographic structure of community seasonality in amphibians, Canavero *et al*. (2009) suggest that both latitude and diversity influence the nestedness and segregation of amphibians across time. Across regions, it is reported that anurans have longer reproductive periods in the wet tropics than those species occurring in tropical seasonal and temperate regions (Duellman & Trueb 1994). In temperate regions, temperature and rainfall, or their interaction, are the primary determinants of reproductive activity within the breeding season, but their relative influence varies by species (Bridges & Dorcas 2000; Oseen & Wassersug 2002; Saenz *et al*. 2006; Steen et al. 2013). In the tropics and subtropics, recent studies have suggested that photoperiod, rather than temperature and rainfall, is the most important predictor of anuran activity (Both *et al*. 2008; Canavero *et al*. 2008; Canavero & Arim 2009). However, even within the wet tropics, the roles of abiotic factors on anuran reproductive activity vary, ranging from not eliciting any response (Inger & Bacon 1968) to a non-random distribution of breeding activity across the rainy season, with abiotic factors such as rainfall being important drivers (Crump 1974; Gottsberger & Gruber 2004). Tropical anurans are also

particularly diverse in their modes of reproduction (Duellman & Trueb 1994), with many species possessing complex oviposition behaviors such as terrestrial or arboreal oviposition, or depositing their eggs in foam nests (Magnusson & Hero 1991; Haddad & Prado 2005). These oviposition strategies are believed to have evolved as a means to reduce exposure of eggs and larvae to predators (Magnusson & Hero 1991). However, these reproductive modes also impose constraints and species need to adjust their calling and breeding activity in accordance with their reproductive mode (Gottsberger & Gruber 2004).

Previous studies indicate multiple drivers influencing the calling activity of anurans across both daily and seasonal time periods. However, many of the studies conducted in tropical or subtropical regions have been conducted over coarse (i.e. monthly or weekly) time scales (e.g., Moreira & Barreto 1997; Both *et al*. 2008; Canavero & Arim 2009; Canavero *et al*. 2009). In this study, I utilized a fine-grain (i.e. daily timeframe) approach to quantify the abiotic correlates of calling activity in an assemblage of tropical anurans occurring in the Gran Chaco ecoregion of south-eastern Bolivia from the middle of the rainy season to the end of the dry season. I describe the pattern of calling phenology that arises from species-specific responses to abiotic factors, specifically I discuss the potential influence of life-history strategies (i.e., reproductive strategy [prolonged vs. explosive breeders] and reproductive mode) on the observed responses to these extrinsic factors.

Materials and Methods

The study was conducted in the semi-arid thorn forests of the Gran Chaco

ecoregion of south-eastern Bolivia. The region has a warm, wet rainy season (November–March) and a cool, dry season (April–October). The surrounding forest in this semi-arid region is predominately thorn forest; common tree species include *Schinopsis lorentzii* and *Aspidosperma quebracho-blanco* with cacti (e.g. *Opuntia* spp., *Cleistocactus baumannii* and *Eriocereus guelichii*) and bromeliads constituting the common understorey plants (Navarro & Maldonado 2002). The study sites were located in one the most xeric regions of the Bolivian Chaco with annual rainfall and temperature averaging 513 mm and 24.6 °C, respectively (Navarro & Maldonado 2002).

Anuran vocalization recordings

I collected audio recordings of nocturnally vocalizing amphibians at seven ponds within the vicinity of the Isoceño community of Yapiroa, one of approximately 25 indigenous communities occurring near the Parapetí River in the indigenous territory of Isoso, Provincia Cordillera, Departmento de Santa Cruz, Bolivia (19.60°S, 62.57°W; WGS 84). I used automated recording devices (SM2+ Song Meters, Wildlife Acoustics, Maynard, Massachusetts, USA), which allow for consistent sampling across extended periods of time (Bridges and Dorcas 2000), to record anuran vocalizations each night. Song Meters were attached to nearby trees within 2 m of the pond's edge (one per pond). The seven breeding ponds that were both artificial (n = 5) and natural (n = 2), and ranged in their hydroperiod from temporary (n = 6) to semi-permanent (n = 1). The Song Meters were deployed at the seven ponds from 20 January 2011 (mid-way through the rainy season) and recorded daily until 31 October 2011 (the end of the dry season). The scope of this study includes data collected from 20 January 2011 to 31 October 2011. Each

Song Meter was set to record for 1 min at the start of each hour starting at 2100 h and ending at 0100 h, for a total of 5 min per night. The recordings were saved to secure digital (SD) cards which were retrieved approximately every 2 wk from each Song Meter. I listened to and transcribed the recordings and identified the vocalizations to species level and the number of calling individuals of each species were estimated. I followed the protocol of Saenz *et al*. (2006) when documenting the number of calling individuals per species per night; when the number of individuals per species calling was ≤4, I felt that I could accurately count the total number of individuals calling; however, when >4 individuals were calling, I assigned a value of 5, as it was impossible to determine the exact number of individuals. Thus, the nightly call intensity index of each species per pond could range from 0 (i.e., no individuals heard) to 25 (summed across five sampling minutes).

Abiotic factors

Previous studies have recognized that anuran calling activity varies across small spatial scales (Oseen & Wassersug 2002; Saenz *et al*. 2006) and similar studies have collected site-specific temperature and rainfall data. I relied on a centrally located weather station sited within the community of Yapiroa as the source for the temperature and rainfall data. The maximum distance between a Song Meter and the weather station was 2 km. The weather station consisted of a HOBO® Data Logging Rain Gauge and a HOBO® Pro v2 External Data Logger (Onset Computer Corporation, Pocasset, Massachusetts). The temperature data logger was covered with an Onset Solar Radiation Shield to block its exposure to direct sunlight. Temperature was measured to the nearest

0.001°C and daily rainfall was measured to the nearest 0.1 mm. I computed the number of hours of daylight using Julian date and -19.6°S latitude for the study sites (Kirk 1994).

Statistical analyses

As in similar studies (e.g., Saenz *et al*. 2006), I observed several anuran species calling several days after a rain event. Therefore, I could not discern whether a species calling activity was an immediate response to precipitation or if it was the result of a lag or build up in precipitation. I used three different types of rainfall lag in the models. The first type was categorical (catlag) and simply indicated the occurrence of rainfall ranging from 1 to 5 d prior to the calling event. For example, catlag1 referenced rainfall 1 d prior to the calling event, catlag2 referenced rainfall 2 d prior to the calling event. The next lag type (rainlag) was the amount of daily rainfall that occurred from one to five nights prior to the calling event. For example, rainlag1 was the rainfall amount from 1 d prior, whereas rainlag2 was the amount of rainfall from 2 d prior to the calling event. The final lag variable type (cumulrain) examined the effects of the cumulative rainfall ranging from 1 to 5 d prior. For example, cumulrain2 equalled the total cumulative rainfall from 1 and 2 d prior to the calling event.

I used logistic regression with generalized estimating equations (GEE) to test the relationship between the occurrence of daily anuran calling activity (0 = no calling and 1 = calling) and air temperature at 21h00, daily rainfall, lags and accumulation in rainfall, and daylength. Because ponds were repeatedly sampled, I used an autoregressive correlation structure. I began with a simple model including temperature and daylength.

I then added rainfall on the day of calling and variables reflecting lags and accumulation in rainfall (five models total). I used QICu to compare models and considered the model with the lowest QICu to be the best model (Pan 2001).

Results

I detected 14 species of anuran at the seven survey ponds (Appendix D). These species did not exhibit much spatial partitioning in their use of calling sites as many species overlapped considerably (Appendix D). Four species, *Dermatonotus muelleri*, *Leptodactylus elenae*, *Leptodactylus fuscus* and *Scinax fuscovarius,* were detected less on than 10 d during the survey period (Appendix E) and were excluded from analyses.

Seasonal and daily call patterns

I observed variation in calling activity across the wet and dry seasons, as the majority of calling activity was concentrated in the rainy season between the months of January and the first week of April (Figure 10, Table 2). However, I detected three species calling in the month of May after a large rainstorm: *Physalaemus biligonigerus*, *Pleurodema guayapae* and *Odontophrynus americanus*. No species was observed calling from June until the beginning of October (Figure 10). At the start of the subsequent rainy season at the end of October I detected *L. fuscus* calling for the first and only time (Table 2).

I observed some variation in calling activity among species within the rainy season (Figures 10 and 11). While the number of individuals calling each night varied, *Leptodactylus bufonius* and *Phyllomedusa sauvagii* called nearly daily during the rainy season (Figures 10 and 11). *Physalaemus albonotatus, P. biligonigerus*, *Rhinella major*

and *Scinax nasicus* were detected every month from January to April, but they called with less frequency when compared to *L. bufonius* and *P. sauvagii* (Figures 10 and 11, Table 1). The calling activity for *Ceratophrys cranwelli* was concentrated towards the beginning of the sampling period (Figures 10 and 11). As previously mentioned, the calling activity of *L. bufonius* and *P. sauvagii* was fairly continuous throughout the rainy season, whereas the calling activity of *P. albonotatus*, *P. biligonigerus*, and to a lesser extent *R. major* and *S. nasicus* were detected consistently for several days at a time (Figures 10 and 11). The calling activity of other species like *C. cranwelli*, *R. schneideri*, *O. americanus* and *P. guayapae*, were detected over much shorter timeframes, often a single night (Figures 10 and 11). There was variation in the calling activity even among these species; *C. cranwelli* calling was concentrated at the beginning of the survey period, whereas the calling activity of other species such as *P. guayapae* and *O. americanus* occurred across a longer time period of the rainy season.

Abiotic correlates of calling activity

Because no species was recorded calling between June and early October, and only three species in late October, I restricted data to 20 January 2011 to 27 June 2011 for the logistic regression analyses. The average temperature at 2100 h during the entire survey period (January–October) was 22.6°C (SD = 5.0 °C, range = 9.4°C–31.2 °C) (Figure 12). The total rainfall recorded during the survey period was 466 mm and the highest amount of daily rainfall recorded was 67.4 mm (Figure 12). The average temperature at 21h00 from 20 January 2011–27 June 2011 was 22.5°C (SD = 4.1°C, range = 9.4°C–30.1°C) (Figure 3). The total rainfall recorded from 20 January 2011–27

June 2011 was 384 mm and the highest amount of daily rainfall recorded was 67.4 mm (Figure 12).

The logistic regression models revealed significant associations between the abiotic variables and calling activity, with each species exhibiting a distinct relationship, but some general trends were also observed (Table 3). With the exception of *O. americanus*, the calling activities of all species were significantly and positively correlated with photoperiod. As another general trend, the majority of species calling were significantly and positively associated with the amount of rainfall on the night of the calling event; the only exceptions were *R. schneideri*, *P. albonotatus* (no association) and *L. bufonius* (significant negative association) (Table 3).

The calling activity of *Phyllomedusa sauvagii* and *Scinax nasicus* were significantly and positively correlated with photoperiod, rainfall on the night of the calling event, and lags in the amount or occurrence of rainfall (Table 3). Calling activity varied slightly between the two species of *Physalaemus,* while each species exhibited similar responses to rainfall and photoperiod, they differed in their response to temperature: *P. albonotatus* calling was significantly negatively associated with temperature, whereas *P. biligonigerus* exhibited no relationship (Table 3). Similarly, the other pair of congeners, the two *Rhinella* species, differed in their calling activity. The main differences being that on the night of calling, *R. major* activity was positively correlated with rainfall and temperature, where *R. schneideri* exhibited no relationship with rainfall, and a significant negative association with temperature (Table 3). *Ceratophrys cranwelli* and *P. guayapae* exhibited the same associations between calling

activity and abiotic variables (Table 3). Calling activity in *O. americanus* was positively associated with amount of rainfall on the night of the calling event, but negatively associated with cumulative amount of rainfall after 4 d (Table 3).

Discussion

Usually generalized as being explosive breeders (Duellman 1999), my study demonstrates that anurans of the Gran Chaco ecoregion span the spectrum of breeding activity of prolonged and explosive breeders (Wells 1977). There was no single abiotic factor that was the dominant driver in calling activity in any of the ten species analyzed; rather I observed that at least two abiotic factors (rainfall and photoperiod) were influencing the calling activity in Chacoan anurans. Similar studies in tropical and subtropical regions found that anurans exhibited a range of responses to climatic factors, ranging from rainfall being the most important variable associated with calling activity to no association between anuran reproductive activity and rainfall and/or temperature (Inger & Bacon 1968; Crump 1974; Gottsberger & Gruber 2004; Kopp & Eterovick 2006). However, some recent studies have identified photoperiod as the predominant driver in reproductive activity in the tropics and subtropics (Both *et al*. 2008; Canavero & Arim 2009). In my study, I found that photoperiod was an important driver of calling activity in nine of the 10 species analyzed (*O. americanus* being the exception) in addition to rainfall and/or temperature. My study design could account for the fact that photoperiod was not the only abiotic variable driving calling activity in the Chaco frog

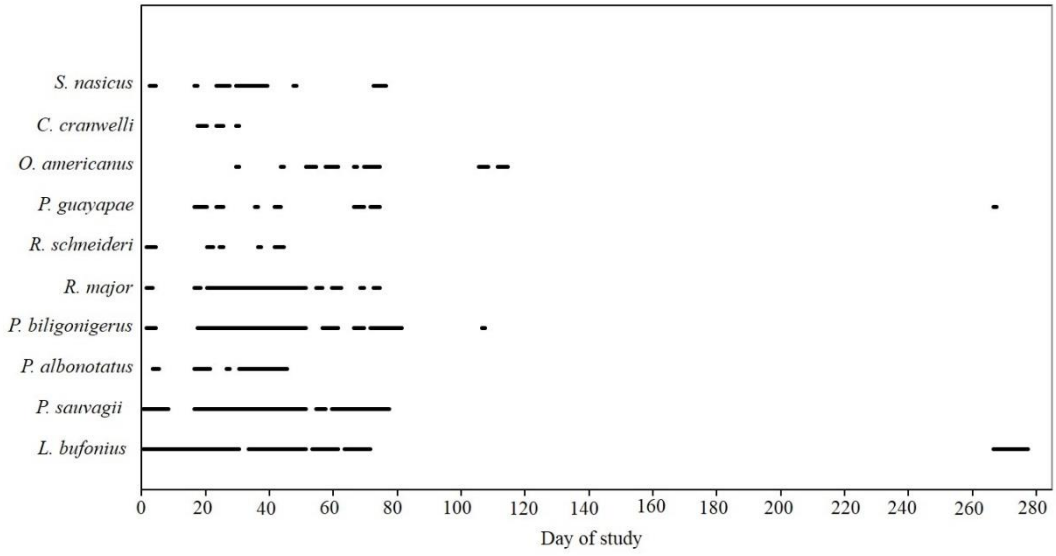

Figure 10. The most frequent calling anuran species recorded over the 285 day study from 20 January 2011 – 31 October 2011 in the Bolivian Chaco. *Leptodactylus elenae*, *Leptodactylus fuscus*, *Dermatonotus muelleri*, and *Scinax fuscovarius* were excluded due to small sample sizes. For species names see Table 1 (Schalk & Saenz 2016).

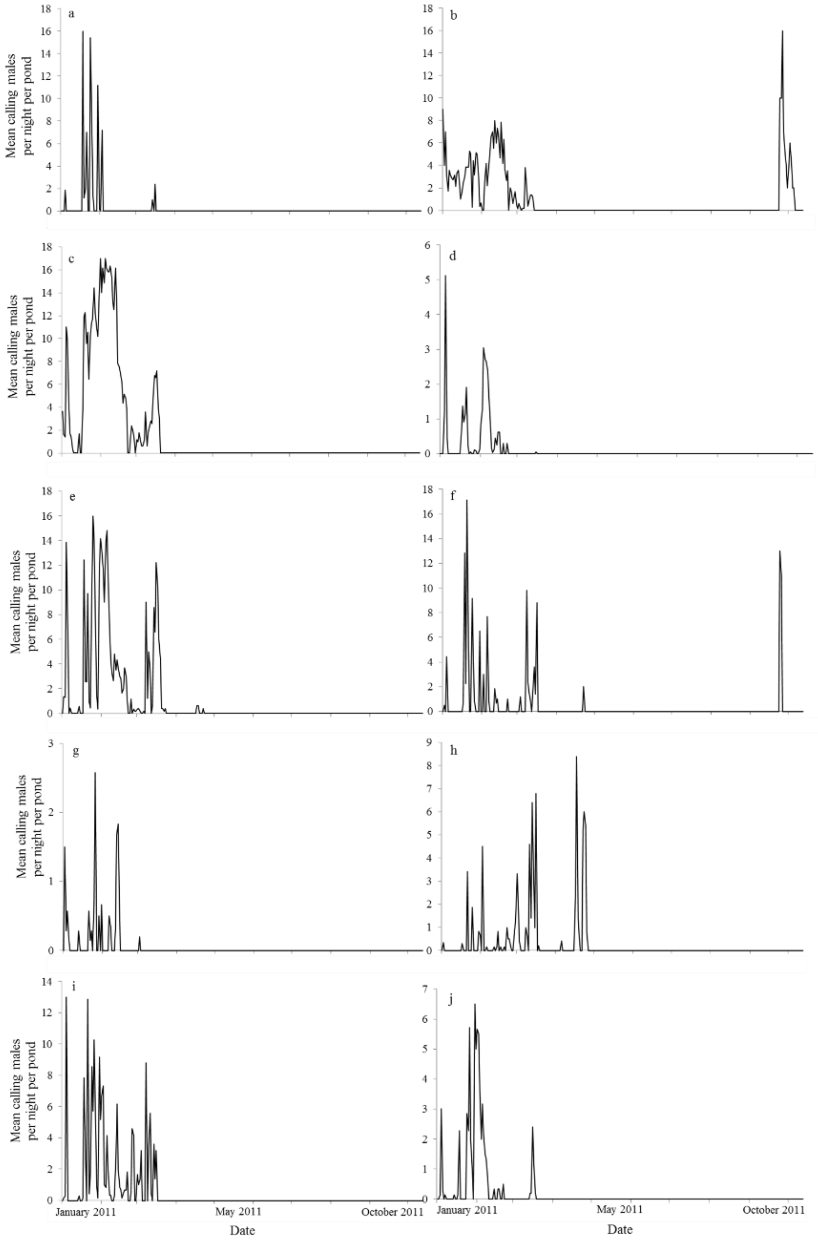

Figure 11. Call intensity scores for 10 species of anurans each night at seven ponds from 20 January 2011 – 31 October 2011: A) *Ceratophrys cranwelli*, B) *Leptodactylus bufonius*, C) *Phyllomedusa sauvagii*, D) *Physalaemus albonotatus*, E) *Physalaemus biligonigerus*, F) *Pleurodema guayapae*, G) *Rhinella schneideri*, H) *Odontophrynus americanus*, I) *Rhinella major*, and J) *Scinax nasicus*. Ponds were located within the vicinity of the Isoceño community of Yapiroa, Cordillera Province, Santa Cruz Department, Bolivia (Schalk & Saenz 2016).

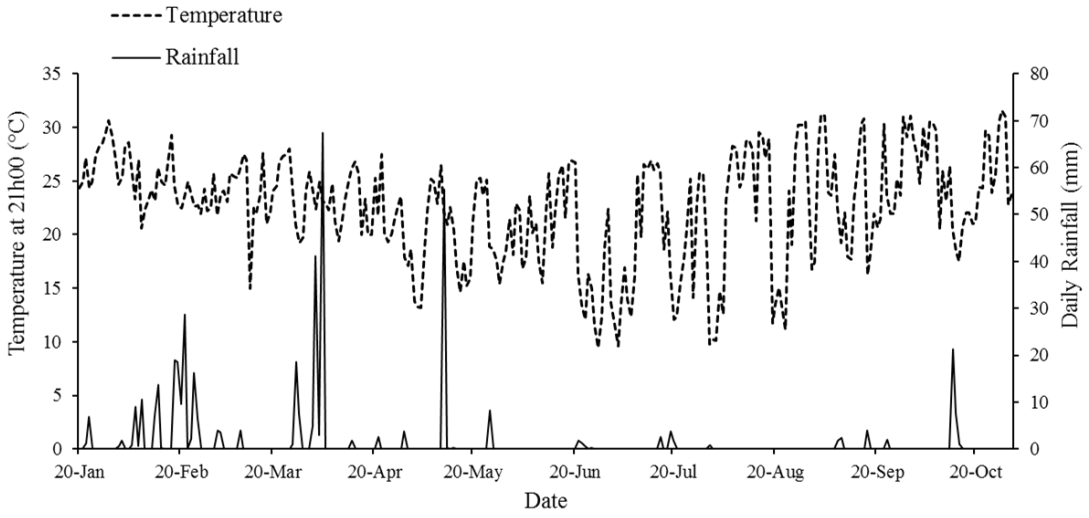

Figure 12. Daily rainfall (mm; solid line) and air temperature at 21h00 (°C; dashed line) collected from a centrally located weather station in the Isoceño community of Yapiroa, Cordillera Province, Santa Cruz Department, Bolivia from 20 January 2011–31 October 2011. Total accumulated rainfall during the survey period was 466 mm (Schalk & Saenz 2016).

Table 2. Calling activity and reproductive modes of 14 species of anurans in the Gran Chaco by month from 20 January 2011 to 31 October 2011. The x symbol indicates that the species was observed calling in the month indicated. Blank cells represent no calling detected in that month for a given species. The species reproductive mode (RM) sensu Haddad & Prado (2005): 1 = Aquatic oviposition and exotrophic, aquatic tadpoles in lentic water (RM 1), 2 = Oviposition in floating foam-nest and exotrophic, tadpoles in lentic water (RM 11), 3 = Oviposition on vegetation above water, exotrophic tadpoles drop into lentic water (RM 24), 4 = Oviposition and early larval stages in foam nest in subterranean nests, subsequent nest flooding, and exotrophic tadpoles in lentic water (RM 30) (Schalk & Saenz 2016).

Family		Month									
Species	RM	January	February	March	April	May	June	July	August	September	October
Bufonidae											
Rhinella major	1	x	x	x	x						
Rhinella schneideri	1	x	x	x							
Ceratophryidae											
Ceratophrys cranwelli	1	x	x		x						
Hylidae											
Phyllomedusa sauvagii	3	x	x	x	x						

Table 2. Continued.

Family		Month									
Species	RM	January	February	March	April	May	June	July	August	September	October
Scinax fuscovarius	1	x	x		x						
Scinax nasicus	1	x	x	x	x						
Leptodactylidae											
Leptodactylus bufonius	4	x	x	x	x						x
Leptodactyluselenae	4	x	x								
Leptodactylus fuscus	4										x
Physalaemus albonotatus	2	x	x	x	x	x					
Pleurodema guyapae	2	x	x	x	x	x					x

Table 2. Continued.

Family		Month									
Species	RM	January	February	March	April	May	June	July	August	September	October
Microhylidae											
Dermatonotus muelleri	1		x								
Odontophryidae											
Odontophrynus americanus	1	x	x	x	x	x					x

Table 3. Results of the best-fit logistic regression models for nine species of anurans in the Bolivian Gran Chaco. The probability of each species calling was modelled with the following variables: 21h00 air temperature, photoperiod, amount of rainfall on the night of the calling event, a lag referencing the occurrence of rainfall ranging from 1 to 5 d prior (catlagX, where X corresponds to the number of days prior to calling), a lag indicating the amount of rainfall occurring from 1 to 5 d prior to the calling event (rainlagX, where X corresponds to the number of days prior to calling), and/or a lag in cumulative rainfall that occurred from 1 to 5 d prior to the calling date (cumulrainX, where X corresponds to the number of days prior to calling). LCL95 = 95% lower confidence level, UCL95 = 95% upper confidence level (Schalk & Saenz 2016).

Species	Variable	Estimate	SE	LCL95 - UCL95	Z	*P*
Rhinella major	Intercept	-18.8	1.19	-21.1 - -16.4	-15.8	<0.0001
	Temperature	0.06	0.01	0.04 - 0.09	5.02	<0.0001
	Photoperiod	1.26	0.11	1.05 - 1.46	11.93	<0.0001
	Rainfall	0.04	0.01	0.03 - 0.06	5.3	<0.0001
	Catlag1	0.98	0.2	0.6 - 1.37	4.99	<0.0001
	Catlag2	0.07	0.16	-0.24 - 0.38	0.44	0.6603
	Catlag3	-0.19	0.15	-0.49 - 0.11	-1.25	0.2104
	Catlag4	0.31	0.13	0.05 - 0.57	2.33	0.0199
	Catlag5	-0.01	0.28	-0.57 - 0.54	-0.04	0.967
Rhinella schneideri	Intercept	-31.0	2.96	-36.8 - -25.2	-10.5	<0.0001
	Temperature	-0.18	0.04	-0.27 - -0.1	-4.2	<0.0001
	Photoperiod	2.66	0.29	2.1 - 3.22	9.27	<0.0001
	Rainfall	-0.04	0.03	-0.1 - 0.02	-1.31	0.191
	Catlag1	0.92	0.28	0.38 - 1.47	3.3	0.001
	Catlag2	-0.72	0.29	-1.28 - -0.16	-2.53	0.0115
	Catlag3	-0.78	0.51	-1.78 - 0.22	-1.52	0.1274
	Catlag4	-0.5	0.41	-1.31 - 0.31	-1.21	0.225
	Catlag5	-0.14	0.38	-0.89 - 0.6	-0.38	0.7054
Ceratophrys cranwelli	Intercept	-83.6	16.2	-115.3 - -51.9	-5.16	<0.0001
	Temperature	-0.34	0.13	-0.59 - -0.1	-2.73	0.0064
	Photoperiod	6.91	1.41	4.15 - 9.66	4.91	<0.0001
	Rainfall	0.19	0.05	0.09 - 0.29	3.72	0.0002
	Catlag1	1.34	0.3	0.76 - 1.93	4.54	<0.0001

Table 3. Continued.

Species	Variable	Estimate	SE	LCL95 - UCL95	Z	*P*
	Catlag2	0.24	0.32	-0.38 - 0.87	0.77	0.44
	Catlag3	-1.53	0.31	-2.13 - -0.93	-5	<0.0001
	Catlag4	2.05	0.34	1.38 - 2.73	5.96	<0.0001
	Catlag5	0.22	0.31	-0.4 - 0.83	0.7	0.4870
Phyllomedusa sauvagii	Intercept	-29.3	3.14	-35.5 - -23.1	-9.33	<0.0001
	Temperature	0.01	0.02	-0.02 - 0.04	0.59	0.5558
	Photoperiod	2.3	0.26	1.8 - 2.8	8.99	<0.0001
	Rainfall	0.02	0	0.01 - 0.03	4.88	<0.0001
	Rainlag1	0.04	0.01	0.02 - 0.06	3.59	0.0003
	Rainlag2	0.03	0.01	0.02 - 0.04	4.29	<0.0001
	Rainlag3	0.03	0.01	0.01 - 0.05	3.7	0.0002
	Rainlag4	0.01	0	0 - 0.01	1.37	0.1716
	Rainlag5	0.01	0	0.01 - 0.02	4.22	<0.0001
Scinax nasicus	Intercept	-33.6	4.98	-43.4 - -23.8	-6.75	<0.0001
	Temperature	-0.06	0.05	-0.17 - 0.05	-1.06	0.2906
	Photoperiod	2.57	0.43	1.73 - 3.41	6	<0.0001
	Rainfall	0.05	0.01	0.03 - 0.07	5.36	<0.0001
	Rainlag1	0.06	0.01	0.03 - 0.09	4.15	<0.0001
	Rainlag2	0.03	0.01	0 - 0.06	2.18	0.0295
	Rainlag3	0.02	0.01	0 - 0.04	1.75	0.0796
	Rainlag4	0.03	0	0.03 - 0.04	7.38	<0.0001
	Rainlag5	0.02	0.01	0 - 0.03	2.08	0.0371
Leptodactylus bufonius	Intercept	-40.0	5.01	-49.8 - -30.2	-7.98	<0.0001
	Temperature	0.1	0.04	0.03 - 0.17	2.94	0.0033
	Photoperiod	3.06	0.38	2.32 - 3.8	8.09	<0.0001
	Rainfall	-0.07	0.02	-0.1 - -0.04	-4.33	<0.0001
	Catlag1	0.12	0.23	-0.33 - 0.56	0.52	0.6036
	Catlag2	0.08	0.2	-0.31 - 0.48	0.4	0.6871
	Catlag3	0.31	0.21	-0.11 - 0.72	1.44	0.1490
	Catlag4	0.03	0.2	-0.37 - 0.42	0.14	0.8859
	Catlag5	0.64	0.08	0.49 - 0.8	8.29	<0.0001

Table 3. Continued.

Species	Variable	Estimate	SE	LCL95 - UCL95	Z	*P*
Physalaemus albonotatus	Intercept	-25.5	3.23	-31.9 - -19.2	-7.91	<0.0001
	Temperature	-0.11	0.02	-0.15 - -0.08	-6.17	<0.0001
	Photoperiod	1.99	0.26	1.47 - 2.5	7.56	<0.0001
	Rainfall	0	0.01	-0.02 - 0.03	0.16	0.8761
	Catlag1	0.61	0.36	-0.1 - 1.31	1.69	0.0907
	Catlag2	1.33	0.23	0.88 - 1.78	5.78	<0.0001
	Catlag3	0.26	0.15	-0.03 - 0.55	1.75	0.08
	Catlag4	0.51	0.24	0.03 - 0.98	2.08	0.0376
	Catlag5	0.29	0.21	-0.12 - 0.71	1.37	0.1707
Physalaemus biligonigerus	Intercept	-18.0	2.37	-22.6 - -13.3	-7.57	<0.0001
	Temperature	-0.01	0.02	-0.06 - 0.03	-0.51	0.6069
	Photoperiod	1.32	0.23	0.86 - 1.77	5.67	<0.0001
	Rainfall	0.04	0.01	0.02 - 0.06	3.26	0.0011
	Catlag1	1.3	0.17	0.97 - 1.64	7.62	<0.0001
	Catlag2	0.68	0.13	0.42 - 0.94	5.05	<0.0001
	Catlag3	-0.08	0.11	-0.3 - 0.14	-0.71	0.4797
	Catlag4	0.84	0.1	0.64 - 1.04	8.17	<0.0001
	Catlag5	0.32	0.18	-0.03 - 0.67	1.81	0.0706
Pleurodema guayapae	Intercept	-24.4	4.76	-33.7 - -15.1	-5.13	<0.0001
	Temperature	-0.15	0.07	-0.29 - -0.02	-2.29	0.0223
	Photoperiod	2.02	0.51	1.01 - 3.02	3.92	<0.0001
	Rainfall	0.09	0.02	0.06 - 0.12	5.7	<0.0001
	Catlag1	1.19	0.19	0.82 - 1.56	6.35	<0.0001
	Catlag2	0.11	0.47	-0.81 - 1.04	0.23	0.8146
	Catlag3	-0.89	0.19	-1.27 - -0.51	-4.62	<0.0001
	Catlag4	1.31	0.19	0.95 - 1.67	7.06	<0.0001
	Catlag5	-0.33	0.24	-0.8 - 0.14	-1.39	0.1636
Odontophrynus americanus	Intercept	-3.91	2.88	-9.55 - 1.73	-1.36	0.1740
	Temperature	0.05	0.03	-0.01 - 0.11	1.58	0.1146
	Photoperiod	-0.03	0.32	-0.66 - 0.61	-0.08	0.9377
	Rainfall	0.07	0.01	0.05 - 0.09	7.35	<0.0001

Table 3. Continued.

Species	Variable	Estimate	SE	LCL95 - UCL95	Z	*P*
	Cumulrain2	0.03	0.02	-0.01 - 0.08	1.54	0.1234
	Cumulrain3	0.02	0.02	-0.01 - 0.06	1.23	0.2189
	Cumulrain4	-0.03	0.01	-0.05 - -0.01	-3.2	0.0014
	Cumulrain5	0.01	0.01	0 - 0.02	1.45	0.1460

assemblages as compared to similar seasonal tropical and subtropical assemblages; sites in those studies were surveyed monthly as compared to nightly monitoring as in my study (Both *et al*. 2008; Canavero & Arim 2009). In some instances, the abiotic variables used varied in the time period over which they were collected; rainfall and temperature were monthly averages, whereas photoperiod was calculated from the day of the survey (Canavero & Arim 2009). I have shown that in most instances amount of rainfall on the night of the calling event, lags in rainfall, and/or daily temperature (along with photoperiod) were important drivers in calling activity for all of the anuran species. As this study highlights, I stress the importance of using abiotic data collected across similar timeframes when attempting to elucidate the factors driving calling phenology in tropical anurans.

The permanency of breeding sites utilized by anurans may also influence their response to abiotic factors. Rainfall tends not to influence calling activity of anurans that utilize semi-permanent breeding ponds, whereas anurans that rely on temporary breeding sites depend on rainfall for the establishment and continued persistence of ponds and to stimulate calling activity (Saenz *et al.* 2006; Steen *et al.* 2013). In the nearby Chiquitano region of Bolivia, rainfall is an important driver of calling activity as several species of anurans that utilized temporary ponds (Schulze *et al.* 2009). All of the Chacoan anurans detected in this study utilize ephemeral ponds as breeding sites which likely explains their significant and positive associations with nightly rainfall or lags in rainfall on previous nights, highlighting that rainfall is an important driver in their calling activity.

There was interspecific variation in the responses to these abiotic factors, and even those species that exhibited similar seasonal activity patterns or those with similar reproductive modes appeared to be responding to different abiotic factors (Gottsberger & Gruber 2004). Two species, *Leptodactylus bufonius* and *Phyllomedusa sauvagii*, had nearly the same pattern in seasonal calling activity; they were prolonged breeders (Wells 1977) across the entire rainy period. The physiology of these two species may provide insights as to their consistent, continuous reproductive activity; *L. bufonius* is one of the few Chacoan anurans with prolonged spermatogenesis occurring almost across the entire year (Cei 1949a). *Phyllomedusa sauvagii* is well-known for its ability to limit its water loss with the waxy secretion that it produces and coats across its skin (Shoemaker *et al*. 1972), thus allowing these frogs to remain active even during the driest periods. Even though these species were similar in their seasonal activity patterns, differences occurred in their daily calling patterns, particularly in their response to rainfall. Calling activity of *L. bufonius* was negatively associated with daily rainfall, whereas *P. sauvagii* showed a positive response. In addition, the calling activity of both species was positively associated with lags in rainfall, but calling in *P. sauvagii* was positively associated with the amount of rainfall from the previous nights, whereas *L. bufonius* called when rain occurred the previous nights, although the amount of rainfall was not important. The difference in the reproductive mode of these two species may provide some explanation to the observed differences in call patterns. Prior to calling, an *L. bufonius* male excavates an underground nest chamber where it breeds with a female and the nest is later capped with mud (Cei 1949b; Reading & Jofré 2003). Once the nest is sealed, the

tadpoles can persist for over 40 d or until heavy rainfall floods the nest allowing the tadpoles to enter a nearby pond (Philibosian *et al*. 1974; Cei 1980; Reading & Jofré 2003). Thus the condition of the mud and the reproductive mode of this species may constrain its daily breeding activity. During nights of heavy rainfall, *L. bufonius* may not be able to construct or maintain the integrity of the nest chamber as it could collapse in on itself and cover recently oviposited eggs with mud; however, the occurrence of rainfall on previous nights may leave the mud still malleable, thereby allowing the male to construct the nest chamber. Newly sealed nests are also vulnerable to predation if the nest breaks apart too soon; Reading and Jofré (2003) observed that newly sealed *L. bufonius* nests that were quickly inundated with water broke down and the egg masses were preyed upon by heterospecific tadpoles. *Phyllomedusa sauvagii* is unique in this assemblage in that it is the only species in the system that oviposits on vegetation overhanging ponds (Perotti 1997). As Gottsberger & Gruber (2004) observed in other species of *Phyllomedusa* that possess the same reproductive mode, calling activity in *P. sauvagii* coincides with high amounts of rainfall occurring across several days, and after the ponds have filled with water. This ensures the chance of the eggs desiccating is low as they develop and that when they finally do hatch, the tadpoles will drop into a well-established pond that has little risk of drying (Gottsberger & Gruber 2004). A similar pattern was also observed in the Chiquitano dry forest of Bolivia, where *Phyllomedusa boliviana* exhibited prolonged calling activity over an artificial pond (Schulze *et al.* 2009).

The patterns of calling activity of *C. cranwelli*, *O. americanus*, *P. guayapae* and *S. nasicus* are indicative of explosive breeders (*sensu* Wells 1977); though they were detected nearly every month in the rainy period, they called only over short periods (i.e. one to several days). *Ceratophrys cranwelli*, *O. americanus* and *S. nasicus* oviposit their eggs directly in the water (Cei 1980; Perotti 1997), whereas *P. guayapae* constructs a flattened foam nest, but its eggs are often exposed even while in the foam nest and vulnerable to predators (Schalk 2012; Valetti *et al*. 2014). Many tadpoles in the tropics have omnivorous or carnivorous feeding habits and are known to prey upon eggs and tadpoles of heterospecifics (Heyer *et al*. 1975; Magnusson & Hero 1991; Altig *et al*. 2007; Schalk *et al*. 2014). Given that the eggs of *C. cranwelli*, *O. americanus*, *P. guayapae* and *S. nasicus* are exposed and vulnerable to predators (Magnusson & Hero 1991), these species may breed over short periods as a means to limit exposure of their eggs to heterospecific tadpoles and other potential predators (e.g., invertebrates [CMS unpubl. data] or killifishes [Montaña *et al.* 2012]) as the pond is colonized by predators over time. While the calling activity of these explosive breeders was positively correlated with daily rainfall, all four species were positively correlated to lags in rainfall, particularly with the day before calling occurred. Explosive breeders tend to utilize ephemeral sites, to which they often need to migrate as the ponds are formed (Wells 1977; Saenz *et al*. 2006). The highly fossorial species can remain inactive for long periods (Cei 1980; Valetti *et al*. 2014), and therefore, they likely need several days of rain to stimulate their emergence and migration to their breeding sites. Similarly, the

hylid *S. nasicus* needs to migrate from their arboreal refugia to their breeding sites, which may explain a similar pattern in its calling activity.

In the lowland tropical forest of French Guiana, Gottsberger & Gruber (2004) observed a temporal partitioning of species breeding and calling activity in accordance to their reproductive mode. If reproductive mode imposes constraints on breeding activity in all anurans, then I would expect to see an emerging pattern where species with the same reproductive mode exhibit a similar response to the abiotic factors, as was observed in Gottsberger & Gruber (2004). However, in this study, congeners with the same reproductive modes exhibited different responses to the abiotic factors in their daily calling activity. The two pairs of congeners occurring in this system (*R. major*/*R. schneideri* and *P. albonotatus*/*P. biligonigerus*), did not partition themselves spatially in their use of calling sites, nor temporally as they overlapped in their calling activity across the latter half of the rainy season. The reproductive modes of some species impose stronger constraints on the reproductive activity as compared to others (Gottsberger & Gruber 2004). Both species of *Physalaemus* and *Rhinella* breed only when a pond has already formed; *Physalaemus* species create floating foam nests that contain eggs, while both species of *Rhinella* oviposit directly in the water. Thus a pond needs to be newly formed or already established for breeding to commence as compared to *L. bufonius*, for example (Cei 1949b). The reproductive modes of *Rhinella* spp. and *Physalaemus* spp. may not impose strong constraints on when these species are able to breed once a pond is formed, which may allow them to exhibit different responses to the abiotic factor and partition calling activity over short time periods, reducing their

overlap. Studies have shown that in other communities, species partition themselves acoustically, spatially and temporally as a means to reduce competition (Crump 1974; Hödl 1977; Duellman & Pyles 1983). It is unclear as to why these species exhibit the temporal partitioning observed. Reproductive character displacement, i.e., the accentuation of differences in courtship behavior in sympatric populations relative to differences in allopatric populations, may provide a mechanism underlying the observed pattern (Brown & Wilson 1956). In anurans, most studies of reproductive character displacement typically focus on differences in mating calls or female mate choice in allopatric and sympatric populations of frogs (Blair 1974; Gerhardt 1994). However, other aspects associated with courtship behavior, such as the selection of calling sites could be explored in this framework; the observed pattern may be the result as a means to reduce competition for calling sites. Höbel and Gerhardt (2003) observed that males of the green tree frog (*Hyla cinerea*) called from higher perch sites when syntopic with the congener *Hyla gratiosa*. In the Chaco, each congeneric pair utilizes the same type of calling site at the breeding ponds; *Rhinella schneideri* and *R. major* call from the edges of ponds (Cei 1980; Schalk & Morales 2012), whereas *P. albonotatus* and *P. biligonigerus* call from the water's surface and among emergent vegetation (Cei 1980; Schalk 2010). Given that there is high overlap in the types of calling sites used by each congeneric pair, suitable calling sites may be limited around breeding ponds. Thus these populations may diverge in the environmental conditions that drive calling activity, allowing the males to segregate temporally and gain access to calling sites that may be otherwise unavailable. To further explore these mechanisms, the calling activity of

allopatric populations of each of the congeneric pair of these species would need to be compared to the syntopic pattern observed in this study. In addition, these patterns highlight that those studies that group and generalize species breeding activity by their reproductive mode (e.g., Gottsburger & Gruber 2004) may not be able to detect the subtle, species-specific differences in breeding activity, especially across short (i.e. daily) timeframes.

This study highlights the importance of weather on regulating the timing of reproductive phenology in this assemblage of tropical anurans. Our understanding of how exogenous (i.e. climatic variables) and endogenous factors (i.e. reproductive modes) interact to influence the temporal partitioning of these species can provide insights on the structure of larval assemblages and the interactions occurring within the breeding ponds (Todd *et al.* 2011). Amphibians exhibit the strongest response to climate warming scenarios by an earlier onset of breeding phenology (Parmesan 2007), thus these results could have implications to understanding potential climate change scenarios and the mistimed species interactions that occur as a result (Saenz *et al.* 2013, Yang & Rudolf 2010; Todd *et al.* 2011). While extreme weather events (e.g., drought) can negatively affect amphibian reproductive activity (Jansen *et al.* 2009), this study suggests even subtle changes in environmental factors, such as an increase in the intermittent period between rainfall, may impact the calling phenology of tropical anurans.

CHAPTER V

PREDATOR-INDUCED PHENOTYPIC PLASTICITY IN AN ARID-ADAPTED TROPICAL TADPOLE∗

Introduction

Phenotypic plasticity is ubiquitous amongst organisms occurring across heterogeneous environments whereby they adjust to environmental variation by producing environmental-specific phenotypes (DeWitt *et al.* 1998; Pigliucci 2001). A wide array of environmental factors, including abiotic factors such as temperature and nutrients, and biotic factors such as competition and predation can induce plastic responses ranging from changes in behavior, life-history, and morphology in diverse taxa (Benard 2004). Predator-induced phenotypic plasticity, in particular, is pervasive across multiple taxa (Tollrian & Harvell 1999), often in the form of morphological or behavioral plasticity (Benard 2004), ranging from development of defences such as spines (Black & Dodson 1990; McCauley *et al.* 2008), reduction in activity (Van Buskirk & Yurewicz 1998), or use of different microhabitats or foraging sites (Petranka 1989; Heithaus & Dill 2002). Larval amphibians, in particular, have served as model systems to examine the trade-offs between predation risk, growth, and costs of

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phenotypically plastic responses (Newman 1992; Van Buskirk *et al.* 1997; Relyea 2001, 2002a, 2002b, 2004; Relyea & Werner 2000).

In many instances organisms exhibit multiple-trait plastic responses across disparate trait types. Given that plasticity occurs when a species experiences environmental variation, certain environments should favor different magnitudes of plasticity for different traits on the basis of their net fitness consequences (Pigliucci 2001). Species inhabiting lentic environments are arrayed along gradients of pond drying and predation (Wellborn *et al.* 1996). Species utilizing temporary ponds are vulnerable to desiccation because of reduced pond permanence, whereas permanent ponds contain a higher abundance and diversity of predators, thus exposing organisms to an increased risk of predation (Wellborn *et al.* 1996). Temporary pond species must maintain the demands of rapid growth and development before the pond dries. To meet the demands of rapid growth and development, these species maintain a high activity level which allows them to garner resources. Because these species must maintain high foraging activity, theory predicts that the predator-induced behavioral traits (i.e., reduction in activity) would come at a greater cost to the organism than predator-induced morphological traits because organisms are still able to maintain high foraging activity while reducing predation risk via morphological traits (e.g., deeper tail fins increase ability to escape) (Richter-Boix *et al.* 2007). As such, temporary pond-adapted species are therefore expected to exhibit predominately morphological predator-induced traits allowing them to reduce predation risk (Relyea & Werner 2000; Anholt *et al.* 2000; Richter-Boix *et al.* 2007), though in extremely ephemeral ponds some species exhibit no

plastic responses in the presence of predators (Dayton & Fitzgerald 2011). Permanent pond adapted species are expected to exhibit both morphological and behavioral predator-induced plastic responses (Richter-Boix *et al.* 2007). These species are not constrained by shortened hydroperiods and behavioral responses, in the form of reduced activity, result in species developing more slowly, but this reduction in activity also reduces the species ability to be detected by predators (Chovanec 1992; Anholt *et al.* 2000; Richter-Boix *et al.* 2007).

When examining the distribution of species along a hydroperiod gradient, one must differentiate between ponds that are temporary as compared to ponds that are variable in pond drying (hereby defined as ephemeral ponds; Perotti *et al.* 2011). Many of the studies on the knowledge of plastic responses of larval amphibians are from temperate species where temporary ponds dry in a predictable fashion annually. Tropical anurans are more diverse in their modes of reproduction than temperate species (Duellman & Trueb 1994; Gomez-Mestre *et al.* 2012), with many species possessing complex oviposition behaviors such as terrestrial oviposition, or depositing their eggs in foam nests (Magnusson & Hero 1991; Duellman & Trueb 1994; Haddad & Prado 2005). One hypothesis is that these oviposition strategies are believed to have evolved as a means to reduce exposure of eggs and larvae to predators by reducing the amount of time spent in aquatic habitats (Magnusson & Hero 1991) (though predation on terrestrial clutches may be just as prevalent [Gomez-Mestre & Warkentin 2007]) as well as protect eggs from desiccation associated with unpredictable pond drying (Crump 2015). While there is an overall evolutionary trend of increasing terrestriality in the life cycle of

anuran amphibians (Gomez-Mestre *et al.* 2012), many species with terrestrial oviposition still rely on aquatic habitats to complete their life cycle (Heyer 1969; Duellman & Trueb 1994; Haddad & Prado 2005). However, less is known concerning behavioral or morphological plastic responses of these tropical species with complex oviposition strategies, as compared to temperate species.

A study that examines how a species responds to different environments with multiple traits can provide insights as to the differences in plasticity among traits, the function of these traits, as well as potential trade-offs among traits (Relyea 2001). Through a laboratory experiment, I tested whether predator chemical cues and alarm cues of injured conspecifics had any effect on development (time to metamorphosis), behavior (activity), or morphology (overall size, body length, tail length, and tail depth) on tadpoles of *Leptodactylus bufonius*, an arid-adapted tropical anuran with terrestrial oviposition. This is a common species in the semiarid Gran Chaco ecoregion of South America with a complex oviposition strategy in terrestrial nest chambers, but the exotrophic larvae complete development in ponds. While this species utilizes ephemeral and temporary ponds, its reproductive mode allows it to complete part of its development outside the constraints of a pond's hydroperiod. Even though *L. bufonius* reproduces in ephemeral and temporary ponds, due to its terrestrial reproductive mode, I hypothesized that tadpoles would present reduced activity, slower development, and deeper tails when exposed to predators

Materials and Methods

The study took place at a park guard camp (Yande Yari) in Kaa-Iya of the Gran

Chaco National Park, Cordillera Province, Santa Cruz Department, Bolivia (S18° 41' 30.516", W62° 18' 6.9474"). The study site is located in the dry Chaco, a habitat characterized by semiarid thorn forest with a distinct rainy season from December – April (see Schalk *et al.* 2013 for further details on the study site). Rainfall averages 512 mm annually (Navarro & Maldonado 2002), but it occurs sporadically during the rainy season, sometimes not occurring for weeks at a time, causing the breeding ponds utilized by the anurans in the region to be highly variable in their persistence on the landscape (Schalk & Saenz 2016). Breeding ponds utilized by *L. bufonius* can range in their hydroperiod from several weeks to months (Cei 1980; Crump 2015; C.M. Schalk, unpublished data, 2010). *Leptodactylus bufonius* is a common, terrestrial anuran of the Gran Chaco that has adapted to the irregular nature of rainfall; calling activity of *L. bufonius* is highest during intermittent periods between rainfall events (Schalk & Saenz 2016). *Leptodactylus bufonius* oviposits terrestrially in a cone-shaped nest chamber that is constructed with mud by the male around the periphery of a breeding pond. The male calls from inside or close to the nests (Crump 1995; Schalk & Sezano 2014). The eggs are deposited in a foam nest after which the entrance is capped off with mud by the female (Philibosian *et al.* 1974; Crump 1995). The eggs hatch in the underground nest chamber after approximately four days (Gosner stage 20; Philibosian *et al.* 1974), but the tadpoles are unable to grow or develop beyond Gosner stage 25 (Gosner 1960) as the biochemical properties of the foam nest inhibits growth (Pisanó 1962). Tadpoles can persist in the nest chamber for over 40 days without water (Philobiosan *et al.* 1974). When the next rainstorm occurs, the nest is flooded and freed tadpoles enter the pond,

where they complete the rest of their development. Tadpoles can metamorphose after approximately 20-30 days in the pond and are members of the generalized, benthic ecomorphological guild (Schalk & Leavitt *in press*).

Phenotypic plasticity experiment

I collected four sealed *L. bufonius* nests that were found around the periphery of ephemeral ponds. The date of oviposition of each nest was unknown, but the developmental stages and sizes were similar across the nests used for the experiment (Gosner stage [Gosner 1960] Average = 25, SD = 0; Total length Average = 10.6 mm, SD = 0.62 mm). During the course of the experiment, tadpoles were fed a fixed diet of 15 mg/tadpole/day of a 3:1 ratio of crushed rabbit food pellets:TetraMin® tropical fish food flakes. The experiment was conducted in a field house, so the ambient temperature fluctuated during the course of the experiment (min. = 22°C, max. = 36°C), but all replicates experienced the same temperature fluctuations and these temperatures are representative of what the tadpoles experience in their natural habitats.

The experiment was a completely randomized design that had three treatments with eight tadpoles per tub. Each treatment was replicated seven times. The three treatments consisted of a predator free treatment and two different predator treatments. Juvenile belostomatid water bugs (*Belostoma* sp., Hemiptera: Belostomatidae) were used as the predators in the experiment. Belostomatids are sit-and-wait predators occurring throughout the study area (C.M. Schalk, unpublished data, 2010) and have been documented as predators of amphibians (Toledo 2005; Schalk 2010). In aquatic habitats utilized by larval amphibians, chemoreception may be the most effective means for prey

to detect and respond to predators and injured conspecifics (Kats & Dill 1998; Saenz *et al.* 2003, Johnson *et al.* 2003). The predators were placed in plastic mesh cages (8 x 8 x 8 cm, mesh size = 2 mm) and had no direct access to the tadpoles in either treatment, but allowed the exchange of water between the cage and tub. The two predator treatments varied in the types of cues to which the tadpoles were exposed; the "non-lethal" treatment contained a caged predator placed at one end of the tub, exposing tadpoles to chemical cues from the predator, whereas the "lethal" treatment consisted of exposing tadpoles to multiple cues and contained a caged predator that was fed one conspecific tadpole daily, thereby exposing the tadpoles to chemical cues from the predator, plus alarm cues from the consumed tadpole. All the belostomatids used were not fed 24 h prior to their use in the lethal treatment. To control for the effect of the cage, the predator-free treatment contained an empty mesh cage, which was equal in size to the cages used in the predator treatments, at one side of the tub. There were some instances where tubs had one or more tadpoles die during the experiment (Predator free = 1, Nonlethal = 2, Lethal = 1). Because this affects the amount of food for each tadpole, which could affect growth and development, these tubs were excluded from analyses. Each plastic tub (34 x 22 x 14 cm) was filled with 3.5 L of well water, which was changed every three days.

To track the morphological changes during ontogeny, I preserved one tadpole from each replicate on four occasions during the course of the experiment (day of experiment: 3, 7, 13, 17). I also documented the Gosner stage (Gosner 1960) of each tadpole collected during the experiment. The experiment ended after 21 days when the

first tadpoles reached Gosner stage 42 (emergence of forelimbs). At the end of the experiment, I measured the remaining four tadpoles in each replicate of each treatment. Those tadpoles that had reached Gosner stage 42 (N = 2) were not included in the final morphological measurements as they undergo rapid morphological changes, including absorption of their tail, at this stage. Using calipers (precision = 0.1 mm), I measured body length, tail length, and tail depth on each tadpole, as these traits often exhibit a plastic response in defence from predators (Relyea & Werner 2000). Prior to examining differences in relative morphology, I adjusted for differences in overall tadpole size by conducting a Principal Components Analysis (PCA) on the three morphological traits and used the score from the first PC axis (PC-1) of each individual as a measure of overall size as the three traits loaded heavily and positively on PC-1 (Relyea & Werner 2000). Each of the three morphological traits were regressed against the PC-1 scores and the residuals were saved (Bookstein 1991). Using the residuals from the morphology data and the log-transformed Gosner stages, I conducted ANOVA and Tukey's HSD to examine differences in morphology and development amongst treatments during each sampling interval. To examine behavioral differences, I measured the activity of the tadpoles amongst each treatment daily by standing approximately 0.5 m away from each tub with my eyes closed, and counted the number of tadpoles moving at the instant I opened my eyes (Skelly 1995). I calculated the proportion of tadpoles moving in each tub to use as the response variable. Because the data were non-normal, I conducted a Kruskal-Wallis to test for differences in activity amongst the three treatments. All data

were log transformed prior to analysis. All analyses were conducted in PAST (Hammer *et al.* 2001).

Results

Morphology

Tadpoles increased in size over time, but tadpoles from both of the lethal and non-lethal predator treatments were significantly smaller than tadpoles from the control treatment on day seven ($F_{2,16} = 12.3$, $p < 0.001$), were nearly smaller on day 13 ($F_{2,15} = 3.31$, $p = 0.065$), but on day 17, only tadpoles in the lethal treatment were significantly smaller than the control treatment ($F_{2,29} = 4.30$, $p = 0.023$), and at the end of the experiment (day 21) tadpoles in the lethal treatment were significantly smaller in size than tadpoles from the control and non-lethal treatments ($F_{2,41} = 11.76$, $p < 0.001$) (as indicated by PC-1; Figure 13a). The morphological responses of the other traits varied during ontogeny across each treatment. Body length fluctuated across treatments during ontogeny; on day seven body length was significantly greater in the absence of predators than in their presence in the nonlethal treatment ($F_{2,16} = 3.74$, $p = 0.046$), whereas on day 13 body length was nearly significantly less in the lethal treatment when compared to the control treatment ($F_{2,15} = 3.55$, $p = 0.055$) (Figure 13b). This differed on day 17 and 21, I observed tadpoles from the non-lethal treatment had greater body lengths when compared to tadpoles from the lethal and control treatments ($F_{2,29} = 4.12$, $p = 0.027$ and $F_{2,41} = 32.08$, $p < 0.001$, respectively) (Figure 13b). No differences in tail length were observed amongst the three treatments until day 17, when tadpoles in the lethal treatment had a greater tail length than tadpoles in the non-lethal treatment ($F_{2,29} = 3.85$, $p =$

0.033). At the conclusion of the experiment on day 21, tadpoles in the control treatment had greater tail length than tadpoles in the non-lethal treatment ($F_{2,41} = 3.25$, $p = 0.049$) (Figure 13c). Tail depth did not differ amongst treatments until the last day of the experiment (day 21); tadpoles in the control and lethal treatments had greater tail depth than tadpoles in the non-lethal treatment ($F_{2,41} = 17.96$, $p < 0.001$) (Figure 13d).

Development

Leptodactylus bufonius tadpoles from the control (i.e., predator-free) treatment developed significantly faster than tadpoles from either predator treatment, which was observed on day seven ($F_{2,16} = 16.4$, $p < 0.001$), day 17 ($F_{2,29} = 3.81$, $p = 0.034$), and day 21 ($F_{2,41} = 11.53$, $p < 0.001$) of the experiment (Figure 13e). Note that at the start of the experiment and on day 13 all tadpoles were the same Gosner stage and thus an ANOVA could not be conducted.

Activity

The tadpoles exhibited a strong behavioral response in the form of reduced activity between the predator and control treatments. Tadpoles were the most active in the control treatment, but were significantly less active when exposed to cues of the predator, and were the least active when exposed to cues of a predator plus alarm cues of a consumed conspecific (Figure 14, Kruskal-Wallis, $H = 50.65$, $p < 0.001$).

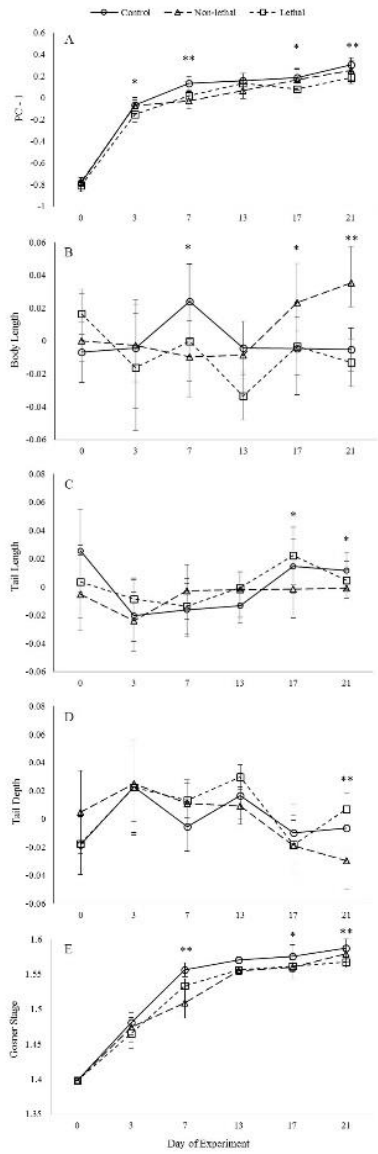

Figure 13. Change in A) overall size (PC-1), relative morphology (mean residuals + SE) of B) body length, C) tail length, and D) tail depth, and E) development (log mean Gosner stage + SE) of *Leptodactylus bufonius* tadpoles across three treatments over the course of a 21 day experiment: the absence of chemical cues (i.e., control treatment, open circles), in the presence of chemical cues of belostomatid juvenile predators (i.e., non-lethal treatment, depicted by open triangles), and in the presence of chemical cues of belostomatid juvenile preadtors plus alarm cues of consumed conspecifics (i.e., lethal treatment, depicted by open squares). Significant differences amongst treatments on a given day are designated by a single asterisk (*) when $p < .05$ or double asterisk (**) when $p < 0.001$ (Schalk 2016).

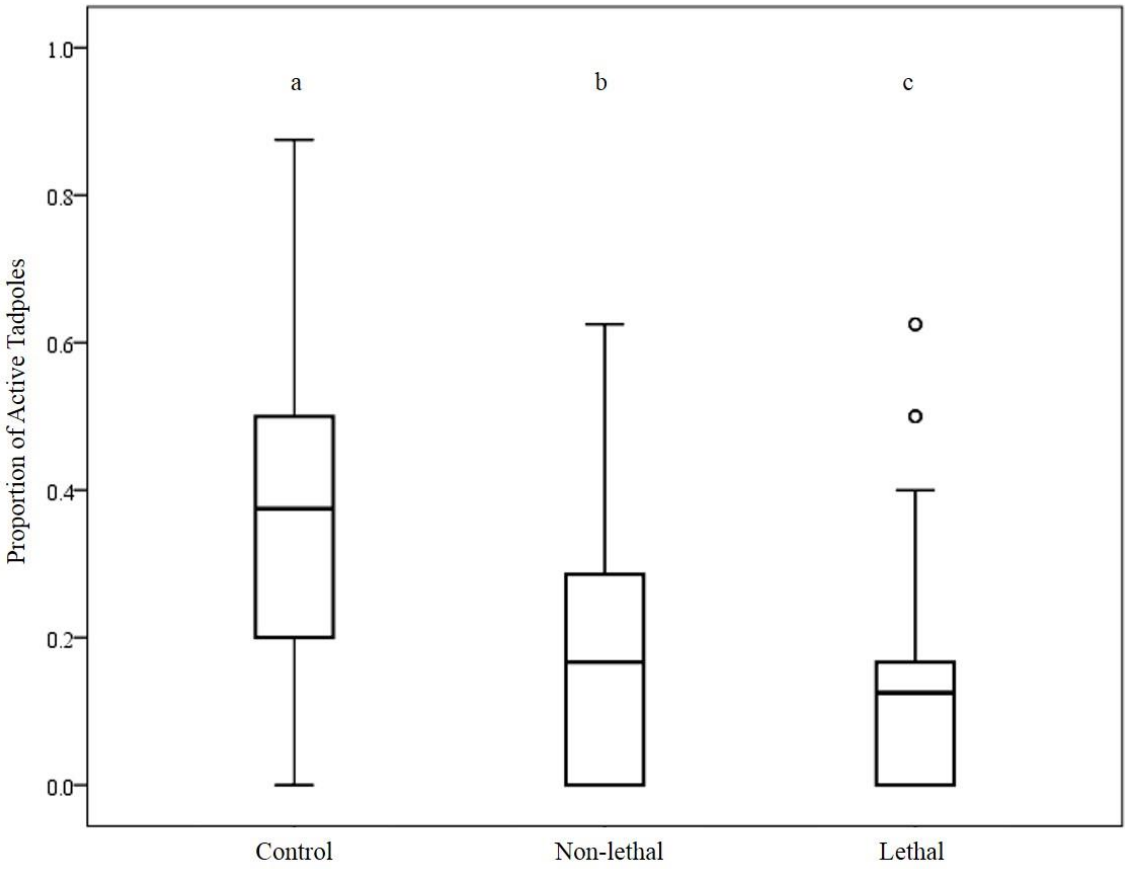

Figure 14. Boxplots of proportion of active *Leptodactylus bufonius* tadpoles across the three experimental treatments. Significant differences in activity level between treatments are indicated by a different letter. Outliers are depicted by an open circle (Schalk 2016).

Discussion

Leptodactylus bufonius exhibited plasticity in behavior, morphology, and development in response to chemical cues from a caged predator and alarm cues from consumed conspecifics. I observed reduced foraging activity under scenarios of increasing predation threat. Coupled with the reduction in foraging activity was a slower growth and developmental rate. Reduced activity levels in the presence of predator and conspecific alarm chemical cues is expected to decrease an individual's risk of predation by reducing the ability to be detected by predators (Azevedo-Ramos *et al.* 1992; Skelly 1994). A trade-off occurs between maximizing food intake and predation risk; an increase in time spent foraging increases the amount of resources acquired, which in turn are available for increased growth, but an increased time spent foraging also increases predation risk (Werner & Anholt 1993). A behavioral trade-off is a mechanism which is believed to influence the distribution of anuran larvae along a gradient of pond permanency (Skelly 1995).

Ontogeny of morphological plasticity

Changes in morphology from predator and injured conspecific cues may be adaptive responses to reduce the risk of predation. Tadpoles generally increase their tail depth while decreasing their tail length in the presence of predators (Van Buskirk & Relyea 1998; McIntyre *et al.* 2004), which has been demonstrated to confer benefits of increased survivorship when exposed to free-ranging predators (McIntyre *et al.* 2004). Studies suggest that increased survivorship is the result of directing attacks towards the tail fin rather than the body (Caldwell 1982, Van Buskirk *et al.* 2003), rather than

improved swimming performance as the effect of these predator-induced tails is small (Van Buskirk & McCollum 2000). Tadpoles in both predator treatments had significantly shorter tails when compared to the predator-free treatment, but tadpoles in the lethal treatment had deeper tail fins than the tadpoles in the non-lethal and predator-free treatment. These changes in larval morphology can have important ramifications for interspecific and intraspecific interactions through changes in functional performance on an individual. Generally predator-induced changes occur early in ontogeny and disappear later (Van Buskirk & Yurewicz 1998; Relyea & Werner 2000). Interestingly in the present study, differences in the morphological traits associated with higher survivorship in anuran larvae under threats of predation (i.e., tail length and tail depth; Van Buskirk & Relyea 1998) did not emerge until the end of the experiment. As a result of colonization and extinction dynamics associated with pond drying, predators can be patchily distributed across the landscape (Relyea & Werner 2000; Werner *et al.* 2007b), and this is especially prevalent in the Gran Chaco ecoregion where hydroperiod of breeding ponds is highly variable, with ponds drying and refilling multiple times during the rainy season (Schalk & Saenz 2016). Given that *L. bufonius* breeds across the entire rainy season (Schalk & Saenz 2016) and the variability in pond persistence on the landscape can cause the predator communities to be patchily distributed across space and time, tadpoles in these variable hydroperiod ponds may employ a strategy to delay inducing plastic responses to ensure that the predation threat is constant while the pond drying threat is minimal.

Tadpoles exhibit context-dependent phenotypic responses under different environmental conditions of predation risk and competition (Peacor & Werner 2004; Relyea 2004; Michel 2012) as well as adapt to the local conditions in their natal ponds, which has been attributed to localized selection of the predator and competitor regimes (Relyea 2002b). In Neotropical tadpoles, shifts in color and morphology have been observed according to predator type (Touchon & Warkentin 2008). Wild-caught *L. bufonius* tadpoles have been observed with tails containing dark melanophores (Schalk & Leavitt *in press*), suggesting that tail color may be another predator-induced phenotypic response in this species, though it was not measured in this study. The belostomatid predator used in this study employs a sit-and-wait foraging strategy (Kopp *et al.* 2006), but *L. bufonius* tadpoles may exhibit a different response when exposed to different predators that use alternative foraging tactics (e.g., active foragers). Other common potential predators in this region include carnivorous tadpoles of *Lepidobatrachus* spp. and *Ceratophrys cranwelli* (Schalk *et al.* 2013; Schalk *et al.* 2014a), other predatory invertebrates such as dragonfly larvae (Odonata; C.M. Schalk, unpublished data, 2010), as well as annual killifishes (Montaña *et al.* 2012; Schalk *et al.* 2014b).

Complex oviposition strategies and phenotypic plasticity of anuran larvae

Many tadpoles that occur in ephemeral or temporary ponds exhibit a strong morphological predator-induced plastic response while still maintaining high activity levels to garner resources (Anholt *et al.* 2000; Richter-Boix *et al.* 2007). However, I observed morphological and behavioral predator-induced plastic responses in tadpoles of

L. bufonius, a species distributed in ephemeral and temporary ponds along the hydroperiod gradient. Species with terrestrial oviposition that utilize ephemeral and temporary ponds, like *L. bufonius*, are able to enter a newly formed pond with a head start in development (up to Gosner stage 25; Philibosian *et al.* 1974; Reading & Jofré 2003) and may be able to afford reduced growth rates via a reduction in activity because the risk associated with a shortered hydroperiod (i.e., risk of pond drying) may be reduced. Philibosian *et al.* (1974) noted that tadpoles of *L. bufonius* hatch in the nest after approximately four days (Gosner stage 20). *Leptodactylus bufonius* tadpoles can spend only 20-30 days in the pond developing, and they are able to get a considerable head start in their development given how short their larval period is in their breeding ponds. Compare this to an aquatic ovipositing species (e.g., most temperate species) that has a similar developmental period that occurs in a pond of the same hydroperiod; risks of mortality because of pond drying for an aquatic ovipositing species may be greater because all stages of development need to occur while in the breeding pond. Therefore, an aquatic ovipositing species may not be able to exhibit strong behavioral responses to predation (i.e., reduced activity) as compared to a terrestrial ovipositing species because it may be at higher risk for mortality as a consequence of pond drying compared to a species that has a considerable head start on development.

The paradigm of community structure in aquatic lentic habitats predicts that the costs and benefits of phenotypic plastic responses of amphibian larvae occur along a hydroperiod and predator gradient (Wellborn *et al.* 1996; Richter-Boix *et al.* 2007). However, this theory has emerged from studies that have utilized temperate species with

primarily aquatic oviposition where eggs are deposited directly in the water (Wellborn *et al.* 1996). Predation and pond drying are still important factors organizing tropical pond communities (Heyer *et al.* 1975; Hero *et al.* 1998; Azevedo-Ramos *et al.* 1999), and the distribution of Amazonian tadpoles across ponds is reflected in their anti-predator traits (e.g., palatability to predators) (Hero *et al.* 2001). Reproductive modes may also influence assemblage structure (Crump 2015). Thus, in addition to the predator and hydroperiod gradients, examining the responses of predator-induced plastic responses along a "terrestriality gradient" would provide insights as to the costs and benefits of increasing terrestriality in anuran reproductive modes on phenotypic plastic responses. For those species that are able to complete part of their development outside of a breeding pond (i.e., species with terrestrial reproductive modes), the pond's hydroperiod may not impose as strong a constraint in a species response to predators as compared to an aquatic ovipositing species with similar life-history attributes (e.g., use of same breeding sites, larval periods of similar duration). Species in the genus *Leptodactylus*, in particular, seem like model organisms to explore the costs and benefits of increasing terrestriality; species range in their degrees of terrestriality ranging from relying on an existing waterbody to oviposit a floating foam nest, to reproducing in the absence of water in terrestrial nest chambers (e.g., *L. bufonius*) (Heyer 1969). For example, in a scenario where different species of *Leptodactylus* with different reproductive modes overlap in their use of breeding sites, those species that oviposit in nests that float on top of the water (e.g., members of the *ocellatus* and *melanonotus* species groups; Heyer 1969) may not exhibit as strong as a behavioral response to predators as compared to

species that oviposit in a terrestrial nest chamber (e.g., members of the *fuscus* species group; Heyer 1969) as the pond's hydroperiod may impose stronger constraints on the species of the *ocellatus* and *melanonotus* species groups as compared to the members of the *fuscus* species group. Given the high diversity and pervasiveness of complex oviposition strategies in the tropics (Haddad & Prado 2005), couching these hypotheses in a comparative framework would provide insights regarding the relative influence of oviposition strategies on interspecific variation in phenotypic plastic responses to environmental stressors.

CHAPTER VI

ONTOGENETIC SHIFTS IN AMBUSH SITE SELECTION OF A SIT-AND-WAIT PREDATOR, THE CHACOAN HORNED FROG (*CERATOPHRYS CRANWELLI*)∗

Introduction

Body size is the single most important trait influencing energetics, life history, and ecology (Peters 1983; Schmidt-Nielsen 1984). Body size influences the breadth of resource utilization, which in turn affects species interactions and their response to the abiotic environment (Wilson 1975; Peters 1983; Werner & Gilliam 1984). Most animals undergo drastic changes in body size as free-living individuals during ontogeny. Because of the wide variation in body sizes of many animal species, the ecological niche of a single species is expected to vary for different body size classes, with multiple size classes occupying distinct ecological niches (Werner & Gilliam 1984; Mittelbach *et al.* 1988; Subalusky *et al.* 2009). Ontogenetic niche shifts are characterized by changes in morphology, resource use, and habitat that occur in concert (Werner & Gilliam 1984).

The most frequently studied aspects of ontogenetic niche shifts in vertebrates are relationships between size, diet, and habitat (Stamps 1983; Werner & Gilliam 1984, Winemiller 1989; Olsen 1996; Byström *et al.* 2012). The selection of habitat sites by an individual is a product of many interacting factors including morphological and

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physiological constraints. Patterns of habitat selection by individuals reflect access to resources (e.g. food), risks of competition and predation, and any number of ecophysiological factors (Eskew *et al.* 2009; Schalk *et al.* 2010). As body size increases during ontogeny, importance and strengths of these interacting factors shift, resulting in ability of individuals to utilize different habitats and food resources. Because of the interaction between habitat and prey resources (i.e., different prey are available in different habitats), shifts in these aspects of the niche occur in concert. Documenting the patterns of ontogenetic shifts in habitat allow one to infer how size-specific selection pressures may drive patterns of resource use, as well understand the ecological roles of predators, competitors, and prey (Stamps 1983). In gape-constrained predators for example, change in size of gape during in ontogeny affects the size and type of prey that can be consumed (Christian 1982; Woolbright & Stewart 1987; Winemiller 1989; Montaña *et al.* 2011).

The anuran life cycle has one of the most distinct and remarkable ontogenetic niche shifts in all vertebrates, where the aquatic larva metamorphoses into a carnivorous, terrestrial adult (Wilbur 1980). Many species of anurans undergo an increase in body size of several orders of magnitude as free-living frogs (Werner 1986), and it is expected these organisms experience significant variation in their ecological niche. Given that frogs can constitute a significant portion of the animal biomass in some ecosystems (Gibbons et al. 2006), and serve as both predators (Wells 2007) and prey (Toledo *et al.* 2007) at multiple age classes, understanding their patterns of resource use can have important implications for their population regulation as well as community structure

(Werner & Gilliam 1984; Werner & Hall 1988; Olson 1996). While the ontogenetic niche shift during metamorphosis has received considerable attention (Wilbur 1980; Werner 1986) there are surprisingly few studies of post-metamorphic ontogenetic niche shifts in anurans. There is some evidence documenting ontogenetic niche shifts in post-metamorphic anurans, especially in diet (Christian 1982; Woolbright & Stewart 1987; Lima 1998; Lima & Magnusson 1998; Hirai 2002), and less frequently, habitat use (Freeland & Kerin 1991). The studies focused on diet have generally found that during ontogeny, anurans change in the type of prey consumed as well as their size, with larger individuals consuming larger prey (Hirai 2002; Lima & Magnusson 1998), which was attributed to the change in both habitat use and gape size, an important predictor in the prey a frog is able to consume (Emerson 1985). However, not all species of post-metamorphic anurans exhibited ontogenetic shifts; Lima and Magnusson (2000) only observed three of the six species of Amazonian leaf litter frogs exhibiting an ontogenetic change in foraging activity. The ontogenetic shifts in habitat use of *Rhinella* [*Bufo*] *marina* L., 1758 have been attributed to the physiological constraints of metamorphs; their smaller body size made them more susceptible to water loss and thus they were often encountered close to the edge of the breeding pond (Freeland & Kerin 1991). In this study, I sought to explore the ontogenetic niche shift in habitat selection, specifically of ambush sites, in a sit-and-wait predator, the Chacoan Horned Frog (*Ceratophrys cranwelli* (Barrio, 1980)). Frogs of the genus *Ceratophrys* are characterized by a very large gape, an extreme sit-wait foraging mode, and cryptic behavior (Schalk *et al.* 2014). They are well known opportunistic predators that consume a variety of invertebrate and

vertebrate prey (Schalk *et al.* 2014). As this species undergoes a nearly four-fold increase in body size during ontogeny of the post-metamorphic life-stage, I predicted adult and metamorph frogs would differ in their general patterns of ambush site. I also predicted adult and metamorph frogs would select ambush sites in nonrandom fashion, based on identifiable habitat characteristics.

Materials and Methods

Study species and study site

Ceratophrys cranwelli is a relatively large frog endemic to the Gran Chaco ecoregion of Argentina, Bolivia, Brazil, and Paraguay (Cei 1980). Like other species of ceratophryids, *C. cranwelli* possesses a gape width > 50% of their body length. Both metamorphs and adults of this species are thus capable of consuming large prey in relation to their body size, and relatively larger prey compared to other anurans (Scott & Aquino 2005; Schalk 2010; Schalk *et al.* 2014).

The Bolivian Gran Chaco, located in southeastern Bolivia, contains large expanses of semiarid thorn forest (Navarro & Maldonado 2002). Rainfall in the region of the study site (average rainfall = 513 mm) is seasonal, occurring between November to March (Navarro & Maldonado 2002). The study site was located in the Isoceño community of Yapiroa (19.60721°S, 62.57492°W), one of approximately 25 indigenous communities distributed along the Parapetí River in the indigenous territory of Isoso.

Frog surveys

I conducted nightly surveys for *C. cranwelli* from 8 March 2009 – 29 March 2009 and from 22 April 2009 to 25 April 2009 (N = 26 nights). Each night, I canvassed

the area around and between 15 breeding ponds (ephemeral: $n = 14$, semi-permanent: $n = 1$) from 2000 h until 0000 h. The distance between ponds was 50 to 400 meters and ponds ranged in size from 40 m^2 to >1000 m^2. I randomized the order of the ponds surveyed each night. I assumed all frogs encountered were in the ambush position as I never encountered any individuals that were moving, calling males, or amplectant pairs.

When a frog was encountered, I estimated microhabitat variables in a 0.25 m^2 quadrat, divided into 25 equal 0.01 m^2 squares, centered on the frog's location. At the same time (within minutes) I also measured the same variables at a randomly chosen, paired, unoccupied site. This paired approach allowed us to test for microhabitat selection among individuals at the appropriate scale of observation (Beck & Jennings 2003; Fitzgerald & Nelson 2011). At occupied and random sites I measured percent cover of plants, woody debris, water, wet mud, and dry mud. I defined wet mud as being malleable when pressure was exerted on the surface, whereas dry mud was not. I also recorded the distance to nearest suitable cover (e.g. logs, or plants), distance to the pond edge, and depth of the water to the nearest cm. I classified encounters according to whether the frog or random site was within or outside the pond. When a frog was found within a pond, it was assigned a negative value and a positive value when it was encountered outside the pond.

A random point could fall anywhere along the cardinal directions from 0.25 to 2 meters from the occupied site. The cardinal direction was chosen at random, and the distance from the occupied site was chosen with a random number generator (the @Rand function in Microsoft Excel™). There was an equal chance random points could

be closer to or farther from the pond edge with respect to the site occupied by that specific frog. The random points occurred in a space that could have been selected by that frog, at the time of observation, and was a sample of a site the individual could have selected, but did not at that moment in time and space. No mean difference between occupied and random variables would mean no habitat selection. This method is informative because it focuses on individual microhabitat selection. In contrast, randomly selecting a sample of points in the study area would bias the dataset towards places that were out of the realm of feasible choices for the individuals I observed. This approach allows to match comparisons of occupied and random sites with the scale of microhabitat selection of the individuals.

I measured SVL (to the nearest mm using dial calipers) of each frog captured. To identify shifts in microhabitat selection that indicate an ontogenetic shift, I calculated the running mean of the frog's SVL against the values of the habitat variables using a lag of three. Frogs immediately released, except a subset (n = 15) that was collected and deposited in the Herpetology Collection in Museo de Historia Natural, Noel Kempff Mercado in Santa Cruz de la Sierra, Bolivia.

Statistical analyses

Using museum specimens, I analyzed allometry of gape width by log-transforming the data and regressing gape width to SVL. Habitat data were not normally distributed and standard transformations failed to normalize distributions and homogenize group variances. Therefore I used non-parametric univariate and multivariate statistics to analyze patterns of habitat selection. I tested for differences in

habitat variables for adult and metamorph frogs, without inclusion of the random points, using a Mann-Whitney U test. To test for ambush site selection in adults and metamorphs, I performed Wilcoxon paired-sample tests on microhabitat variables for ambush sites and nearby paired random sites. To test for multivariate microhabitat selection, I conducted a one-way Analysis of Similarity (ANOSIM; Bray-Curtis distance; permutations = 9999) on occupied and random sites for adults and metamorphs, ANOSIM is a nonparametric alternative to MANOVA that does not depend on multivariate normality of the data (Gotelli & Ellison 2004). ANOSIM produces a statistic, R, that indicates the magnitude of difference among groups. An R = 1.0 indicates that assemblages are completely different, and R = 0.0 would reflect complete similarity (Clarke & Warwick 1994). All other analyses were conducted in the program PAST (Hammer *et al.* 2001). To further evaluate patterns of habitat use, I calculated non-metric multidimensional scaling (NMDS) analyses of all frogs combined and for adults and metamorphs separately. The NMDS ordination was constructed using presence/absence data by location of adult and metamorphs, adults and their respective random points, and metamorphs and their respective random points. Non-metric multidimensional scaling is well suited for this purpose because it is unconstrained by environmental variables (McCune & Grace 2002). I could then fit environmental variables to the ordination as vectors and identify important environmental gradients. The ordination was completed using Bray-Curtis distance measure in PC-Ord (McCune & Mefford 1999). In NMDS, the robustness of the ordination is indicated by its stress value: < 0.2 give a potentially useful two-dimensional picture, stress < 0.1 corresponds

to a good ordination and stress < 0.05 is an excellent representation (Clarke & Warwick 1994).

Results

I captured 96 individuals and none were recaptures. The running means of distance to pond's edge and proportion of dry mud shifted distinctly at 42 mm SVL (Figure 15). Frogs smaller than 42 mm SVL were found in a wide range of distances to pond's edge, with a preponderance of small individuals relatively far from the edge, and on dry mud.

These results gave us an ecological basis for classifying the frogs as metamorphs (<43 mm SVL) and adults (>42 mm SVL). The 42 mm size break is also consistent with the upper SVL of first year metamorphs in *C. cranwelli* (generally between 25-42 mm) (Fabrezi 2011; pers. obs.) and adults (those frogs with an SVL greater than the range of SVL values of metamorphs) (Figure 16). Gape width of *C. cranwelli* was significantly correlated with SVL, and exhibited a slightly negative pattern of allometric growth in relation to SVL ($y = -.13747 + 0.95362x$, $p < 0.001$). I only found *C. cranwelli* near ephemeral ponds; no individuals were found around the semi-permanent pond or between ponds. The NMDS axes 1 and 2 accounted for 72% of the variation (Axis 1: 57.1%; Axis 2: 14.9%) and had a stress value of 0.12, indicating the ordination was informative (Figure 17a). ANOSIM revealed that adults and metamorph *C. cranwelli* occupied different microhabitats (Figure 3A, $p = 0.0029$, $R = 0.1842$). Metamorphs occurred significantly further from the pond's edge (Mann-Whitney, $U = 592$, $p = 0.01086$) and occupied areas with a higher proportion of dry mud compared to adults

(Mann-Whitney, $U = 571.5$, $p = 0.001698$), whereas adults were found in sites with a higher proportion of water (Mann-Whitney, $U = 550.5$, $p = 0.0003371$) and in deeper water (Mann-Whitney, $U = 655.5$, $p = 0.00029$).

The NMDS ordination of the adult *C. cranwelli* and their respective random points resulted in two axes accounting for 68.6% (Axis 1: 56.6%; Axis 2: 12.0%) of the variation, with the ordination considered to be good with its stress value of 0.09 (Figure 17b). Adults generally, exhibited no selection in their ambush sites when compared to their respective random points (Figure 17b, ANOSIM, $p = 0.1005$, R = 0.03729). The Wilcoxon paired-sample tests revealed the percentage of dry mud was the only microhabitat variable that was significantly different between the adult and random points ($p = 0.03$, $z = 2.218$); adults avoided areas with a higher proportion of dry mud, when compared to their random points.

The NMDS ordination of the *C. cranwelli* metamorphs and their respective random points resulted in two axes accounting for 61.2% (Axis 1: 46.6%; Axis 2: 14.6%) of the variation, and was considered to be useful having a stress of 0.12 (Figure 17c). Metamorphs selected a smaller proportion of the available habitat to use as ambush sites (Figure 17c; ANOSIM, $p = 0.0001$, R = 0.1051). Wilcoxon paired t-tests revealed metamorphs selected points significantly further from water than the random points ($p = 0.017219$, $z = 2.382$). Metamorphs selected areas with a significantly higher proportion of dry mud ($p < 0.0001$, $z = 4.697$) and occurred significantly less in areas with a higher percentage of water cover ($p = 0.003$, $z = 3.147$) and water depth ($p < 0.0001$, $z = 4.365$).

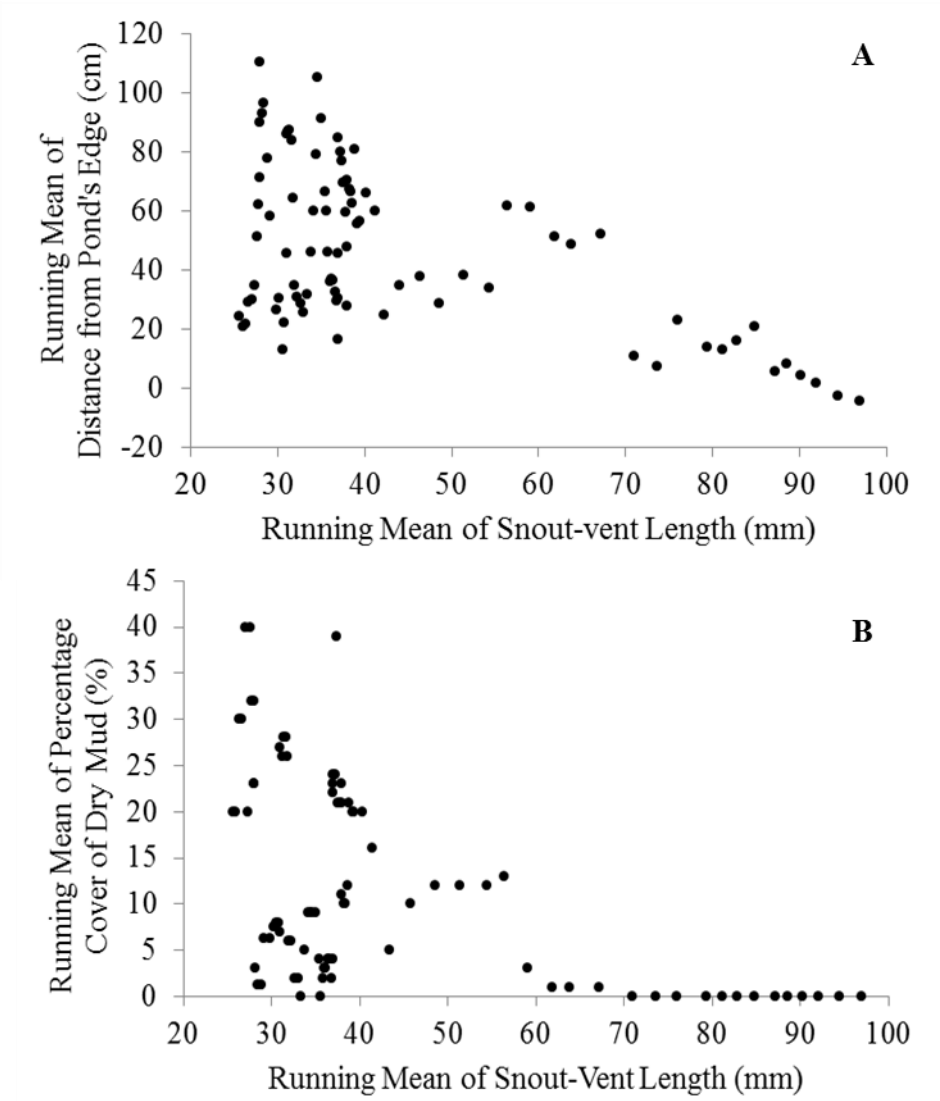

Figure 15. Running mean of A) SVL vs. distance from the pond's edge and B) SVL vs. percentage cover of dry mud (Schalk & Fitzgerald 2015).

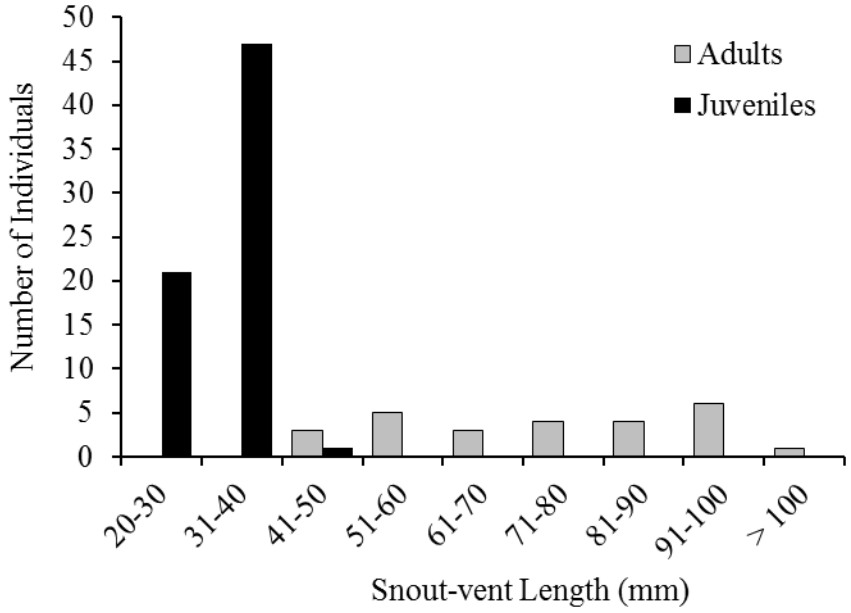

Figure 16. Size-class distribution of the metamorph (black bars) and adult (grey bars) Chacoan Horned frogs (*Ceratophrys cranwelli*) (Schalk & Fitzgerald 2015).

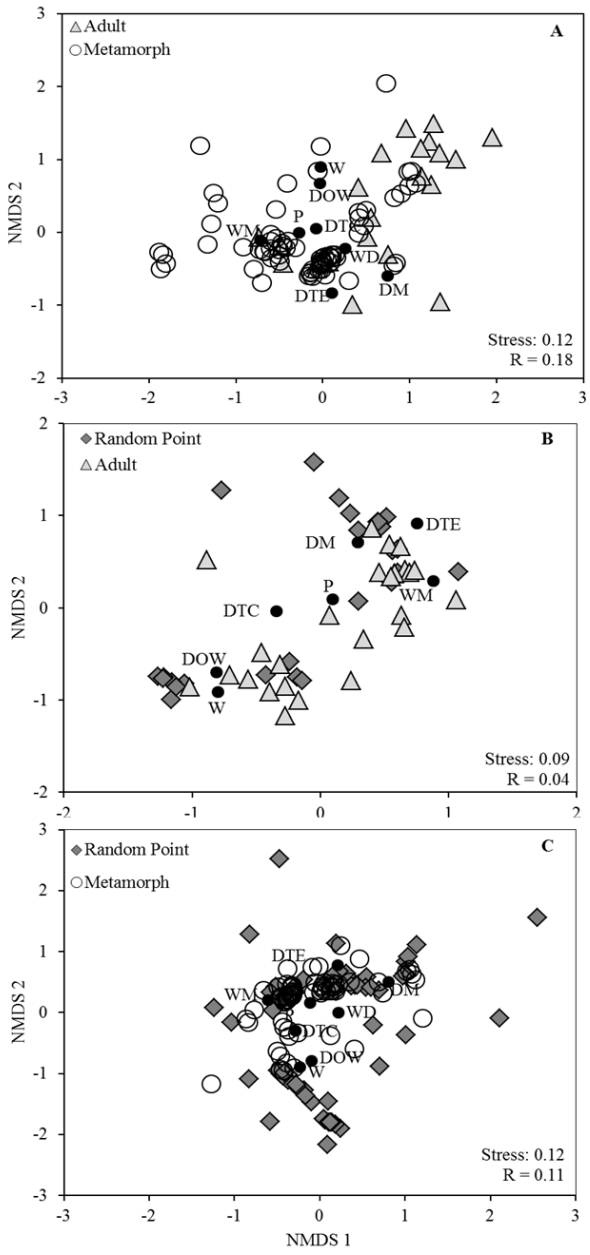

Figure 17. Non-metric multidimensional ordination of *Ceratophrys cranwelli* A) metamorphs and adults, B) adults and their respective random points, and C) metamorphs and their respective random points in relation to the environmental variables (black circles) measured. Abbreviations of the environmental variables are as follows: DTE = distance of edge of pond, DTC = distance to cover, WM = percent cover of wet mud, DM = percent cover of dry mud, W = percent cover of water, P = percent cover of plants, WD = percent cover of woody debris, DOW = depth of water. The R value is the R statistic produce from the ANOSIM (Schalk & Fitzgerald 2015).

Discussion

Selection of foraging sites is the product of a tradeoff driven by food availability and the cost, or perceived cost, concomitant with those sites (Werner *et al.* 1983; Lima & Dill 1990; Eskew *et al.* 2009; Schalk *et al.* 2010). In this study, I observed an ontogenetic niche shift in the form of ambush site selection, between metamorph and adult *C. cranwelli*. At the broader habitat scale, I observed a pattern where both niche classes of *C. cranwelli* were associated with ponds. Duellman and Lizana (1994) studied the ecology of a congener, *Ceratophrys cornuta* L., 1758, in the Peruvian Amazon, and they did not observe ontogenetic shifts in habitat selection in that species, they also observed that *Ceratophrys cornuta* used different types of habitat compared to the *C. cranwelli* in this study. Individuals of *C. cornuta* were found more often in the leaf litter in the surrounding forest and few individuals were found within the vicinity of water, the majority of which were calling males (Duellman & Lizana 1994). Members of the genus *Ceratophrys* are sit-and-wait predators and rely on prey that possess a higher mobility than themselves to increase encounter rates (Huey & Pianka 1981; Taigen & Pough 1983). Additional attributes of prey of sit-and-wait predators include being fewer in number, more solitary, and relatively large in size (Toft 1980). Suitable prey for *C. cornuta* are likely more dispersed and widespread in wet tropical rain forests, which may account for the observed distribution of *C. cornuta* across the entire forest (Duellman & Lizana 1994). In contrast, the xeric nature of the habitat of the Gran Chaco may cause prey to be concentrated around ponds, which may account for the observed ambush site selection in *C. cranwelli*. While this region does have a pronounced rainy and dry season

as most areas in the tropics, this area of the Chaco receives the least amount of rainfall across the entire ecoregion, with corresponding high variation in precipitation (Navarro & Maldonado 2002). It typically may not rain for up to two weeks even during the rainy season (pers. obs.). Thus breeding and foraging habitat for *C. cranwelli* is patchy across the dry Chaco landscape. The prey of *C. cranwelli* may be concentrated around these breeding ponds and likely drive the aggregation of *C. cranwelli* that we observed.

The morphological constraints of a size class also affect the types of food available that can be utilized by an individual in a foraging site (Werner & Gilliam 1984). Studies of trophic ontogeny of anurans have revealed that prey size increases with increasing gape width (Christian 1982; Hirai 2002), though there have been exceptions (Lima 1998). Skull size is an important predictor in the size and type of prey a frog can consume, with those species possessing a wide gape (e.g. *Ceratophrys* spp.) being adapted for consuming large prey such as vertebrates (Emerson 1985). Although data on prey type, size, and number were unavailable for this study, the allometric analysis of gape width provides insights into foraging differences of the two ontogenetic niche size classes. As SVL increased, gape width increased linearly and rapidly with SVL, increasing over five-fold during ontogeny. Allometry of gape width was slightly negative (slope = 0.953), with small metamorphs possessing relatively large gape width. Thus both small and large individuals possess the morphology to consume large prey, but during ontogeny larger individuals are able to consume increasingly larger prey. This species has been observed preying upon vertebrates at both the metamorph and adult life-stage (Schalk *et al.* 2014). Organisms that are gape-limited that undergo a large

increase in body size during ontogeny often exhibit a size-dependent shift in diet (Christian 1982; Werner & Gilliam 1984; Byström *et al.* 2012), and the prey availability is a factor responsible for the observed ontogenetic niche shift. Because there is large difference in the size of the prey that can be consumed between adults and metamorphs, it is unlikely that the metamorph size class would be utilizing similar food resources as the adults.

Selection of foraging sites coincides with high availability of food (Harris 1986; McCormick 1998). The ambush site selection of the adults and metamorphs may be reflective of the spatial and size segregation of the prey of *C. cranwelli*, which includes other species of frogs (Scott & Aquino 2005; Schalk *et al.* 2014). Ponds in the dry Chaco serve as breeding sites by 17 species of anurans at this study site (unpubl. data), and individuals of many species are concentrated around the pond's edge to call, oviposit, or as they emerge from the pond as metamorphs. When compared to the metamorphs, adult *C. cranwelli* utilized ambush sites closer to the pond's edge, and when compared to their random points avoided areas with a high proportion of dry mud. Ambush sites near the pond's edge would increase encounter rates with frog prey (Scott & Aquino 2005; Schalk & Montaña 2011) which coincides with the observations by Schalk *et al* (2014) that documented a high proportion of frog prey in the diet of *C. cranwelli*. However, the sites of the adult frogs were no different from the random sites, which were within two meters of the ambush site. Adult *C. cranwelli* may be able to utilize a relatively broad range of ambush sites close to the pond's edge, which is why the site-selection analysis did not distinguish random sites from the ambush sites for adults. Conversely, the

ambush sites selected by metamorphs was significantly different than the random sites. *Ceratophrys cranwelli* metamorphs are more limited in the range of the size of prey they can consume, and the distribution of smaller prey items consumed by metamorphs may exert a stronger influence as to the ambush sites they select. It has been well documented that juvenile amphibians constitute the dispersal stage for many pond breeding amphibians (Semlitsch 2010). Metamorphs of *C. cranwelli* have been observed consuming metamorphs of other anuran species (Schalk 2010; Schalk *et al.* 2014) and their selection of in sites further from the pond's edge may be in response to encounter rates with small, emerging metamorphs of other frog species that may be dispersing into the terrestrial landscape and other small prey such as terrestrial invertebrates.

Counteracting the ability of an animal's foraging strategy are the morality risks associated with that habitat (Lima & Dill 1990). *Ceratophrys cranwelli* metamorphs selected a smaller subset of the total available habitat, specifically sites that were further from the pond's edge and with a higher proportion of dry mud, whereas the ambush sites utilized by the adults were no different from the random sites. Smaller body sizes are more susceptible to desiccation because of a higher surface area to volume ratio, and these physiological constraints can influence the spatial distribution of an organism (Freeland & Kerin 1991; Eskew *et al.* 2009). In anurans, metamorphs tend to be associated with more mesic microhabitats because they are more vulnerable to desiccation (Freeland & Kerin 1991), yet I observed an opposite pattern in this study. The physiological cost may be low for metamorphs as they are adapted to the arid habitat of the Gran Chaco (e.g., the ability to form cocoons to limit water loss while

aestivating; Cei 1980) thereby allowing them to select and exploit foraging sites that could be unavailable to other anuran species that may be more constrained by their physiology (Freeland & Kerin 1991)

Various forms of intraspecific interactions (e.g., cannibalism, competition for space, avoidance behaviors) between adults and metamorphs could each drive the pattern of microhabitat selection I observed. Displacement interactions between adults and metamorphs is possible, but seems unlikely to be driving the observed pattern. Metamorphs could be at risk of cannibalism by adults near the pond's edge, as an increase in the difference in body size increases the potential for asymmetric predatory interactions (Werner *et al.* 1995). However, cannibalism has not been observed in the post-metamorphic life-stage of this species (Schalk *et al.* 2014) and while cannibalism is assumed to occur frequently in the frogs of the family Ceratophryidae, this overgeneralization appears to stem from very limited data (Schalk *et al.* 2014). Some species of anurans shift in their foraging strategy during ontogeny (Lima 1998), but *C. cranwelli* maintains its sit-and-wait strategy as both metamorphs and adults. Given that species' with this foraging strategy relies on large, mobile, prey (Toft 1980), adult and metamorph *C. cranwelli* may have a low likelihood of encountering one another, as they are both sit-and-wait predators. Thus, metamorphs may select sites that have a low likelihood of encounter rates with adults to minimize any potential for cannibalistic interactions as I observed a disparity in the ambush sites selected amongst the two different size classes. The mechanism driving this pattern could be an evolutionary relict, i.e., "ghost of cannibalism past" (*sensu* Connell 1980) where cannibalism could

have driven microhabitat selection, but cannibalism is now rare in a contemporarily. The alternative hypotheses that microhabitat differences are due to avoidance interactions, or driven by distribution of prey sizes in different microhabitats are not mutually exclusive, and both may be influencing the ontogenetic shift I observed. I did not measure distribution and abundance of prey during this study, and further research would be needed to disentangle the roles of avoidance and prey-based habitat selection in these frogs.

It is unlikely that territoriality explains the differences in ambush site selection between adults and metamorphs of *C. cranwelli*. Territorial behavior, for example, allows for access to critical resources (Kaufmanns 1983), but the primary purpose of territoriality in anurans is associated with reproduction (Wells 1977; Pröhl 2005). I did not observe any reproductive behavior (e.g., calling males or pairs in amplexus) during this study that would indicate the establishment of territories by the adults. Female *C. cranwelli* exhibit aggressive behaviors during courtship and when in amplexus, but it is unknown whether the purpose of such behaviors are due to female mate choice or a type of predatory behavior (Schalk & Montaña 2011; Schalk *et al.* 2014; Silva *et al.* 2014).

This study adds to the scant literature on ontogenetic shifts in the post-metamorph life stage of anurans and provides the first information on ontogenetic differences in habitat selection in this species. Intraspecific and interspecific variation in body size influences the type and strength of ecological interactions (Werner & Gilliam 1984). Habitat selection is the most fundamental and important aspect in understanding a species' niche (Pianka 2000; Morris 2003; Wells 2007) but it is acknowledged that it

may change during ontogeny (Werner & Gilliam 1984). The pattern that arises from studying ontogenetic shifts in habitat selection provides an important foundation to generate and test mechanistic hypotheses to elucidate the ecological factors driving the observed pattern (Werner 1998). Combining this information with diet, activity patterns, as well as prey distribution may allow one to make better predictions as the mechanisms driving the observed niche shifts for post-metamorphic anurans, and be better able to accurately describe and understand the factors influencing their community structure.

CHAPTER VII

ON THE DIET OF THE FROGS OF THE CERATOPHRYIDAE: SYNOPSIS AND NEW CONTRIBUTIONS

Introduction

"They [Intanha; *Ceratophrys aurita*] are fearless and react to any attack, running with their mouths open behind their aggressors. … They can grab everything that is at their reach and that moves; they do not neglect even the chicks as big as volumous as they are." (Miranda-Ribeiro, 1920; translated by H. R. da Silva).

Frogs of the family Ceratophryidae have long fascinated biologists, which has resulted in a body of literature that predates Linnaeus and rivals in size that for other similarly sized groups of frogs (e.g., Seba, 1734; Linnaeus, 1758; Shaw, 1802). The Ceratophryidae is comprised of three genera; *Ceratophrys* is the most species-rich genus with eight species, followed by *Lepidobatrachus* (three species), and the monotypic genus containing *Chacophrys pierottii* (Frost, 2014). *Ceratophrys* is distributed throughout tropical South America, ranging from the Caribbean lowlands of Venezuela and Colombia in the north, and the Pacific coast of Ecuador in the west, southward through Amazonia to the Gran Chaco of central Argentina and eastward into the Cerrado of southeastern Brazil (Lynch, 1982; Frost, 2014). All three species of *Lepidobatrachus* and *C. pierottii*, on the other hand, are endemics to the Gran Chaco ecoregion of central South America (Cei, 1980).

As adults, species of *Ceratophrys* and *Chacophrys pierottii* are more terrestrial than those of *Lepidobatrachus*, which are aquatic and inhabit temporary pools during the rainy season in the Gran Chaco (Cei, 1955; 1958; 1968; 1980). All species of ceratophryids, however, are considered sit-and-wait ambush predators (Duellman and Lizana, 1994; Scott and Aquino, 2005) and their behaviors and morphologies that reflect such a foraging mode have become legendary. All species of ceratophryids, for example, possess a wide gape and fang-like teeth, which are presumed to be traits adapted for consuming large, slow-moving prey such as vertebrates (e.g., Figure 18; Emerson, 1985; Fabrezi and Emerson, 2003).

The tadpoles of ceratophryids are equally as remarkable as the adults. Those of species of *Ceratophrys* and *Lepidobatrachus* possess morphologies indicative of their macrophagous and carnivorous habits (Cei, 1968; Rubial and Thomas, 1988; Altig and McDiarmid, 1999; Wild, 1997a, 1997b; Natale et al., 2011; Wassersug and Heyer, 1988). Tadpoles of the species of *Lepidobatrachus* have some of the most distinctive body morphologies of any anuran larvae, with their wide, dorsoventrally flattened head and lack of keratinized mouthparts, which allow them to engulf prey whole (Cei, 1968; Rubial and Thomas, 1988). The mouths of tadpoles of the species of *Ceratophrys*, on the other hand, are terminal and contain strong keratinized jaw sheaths and keratodonts that facilitate biting and chewing of prey (Wild, 1997a, 1997b; Altig and McDiarmid, 1999; Vera Candioti, 2005). The tadpole of *Chacophrys pierottii*, however, lacks such distinctive traits and has a body plan and morphology similar to that of a Type IV

tadpole (Orton, 1953; Wild, 1999; Quinzio et al., 2006) and is considered a member of the benthic ecomorphological guild (Altig and McDiarmid, 1999).

The literature is replete with comments regarding the carnivorous ambitions of ceratophryid frogs, including cannibalism, at all stages in their life cycle (e.g., Noble, 1931, Parker, 1931; Cei, 1980). However, with a few exceptions (e.g., Basso, 1990; Duellman and Lizana, 1994; Pueta and Perotti, 2013), there is little published data on the diet of most species of the Ceratophryidae, and that which exists is scattered among anecdotal reports (e.g., feeding observations of a single captive tadpole; Miranda-Ribeiro, 1923) or is overly general (e.g., "…feeding on small vertebrates and arthropods…" Cei 1980). Herein I provide a comprehensive synopsis of the current state of knowledge of the diet of these remarkable frogs throughout their life cycle stages from results of a comprehensive survey of the literature, from field observations, and from specimen-based research.

Figure 18. A) An adult *Ceratophrys cornuta* consuming a medium-sized (ca. 50–90 g) oryzomyine rodent (e.g., *Euroryzomys* or *Hylaeamys*) at Reserva Amazonica on the Río Madres de Dios, Departamento de Madre de Dios, Peru (photo by Kyle Salzmann). B) An adult female *Ceratophrys cranwelli* consuming a *Rhinella major* (Anura: Bufonidae) in a temporary pond in the community of Yaprioa, Provincia Cordillera, Departamento de Santa Cruz, Bolivia (photo by C.M. Schalk).

Materials and Methods

Literature review

I surveyed the literature to provide a comprehensive list of known prey items of the species within Ceratophryidae. I used Web of Knowledge and Google Scholar to search for published articles, books, and book chapters using the following keywords: "Ceratophryidae", "*Ceratophrys*", "*Chacophrys*", "*Lepidobatrachus*", "prey", "diet", "food", and "tadpole". Additional references were gathered from searching through the literature cited sections of the sources produced from the database searches. The search included both tadpole and post-metamorphic life stages, under both natural (e.g., field observations or specimen dissections) and captive (e.g., feeding experiments) conditions but only with native prey. Thus, experiments conducted with non-native prey or reports of captive feeding on non-native prey, although intriguing (e.g., a *Ceratophrys* eating an 11 inch *Alligator mississippiensis*; Parsons, 1932), were not included in the survey as they address what these frogs *can* eat. I was interested in what they *do* eat in nature.

Specimen dissections

Twenty-one individuals from seven species of ceratophryids were dissected: *Ceratophrys aurita* (n = 3), *Ceratophrys calcarata* (n = 2), *Ceratophrys cranwelli* (n = 2), *Ceratophrys ornata* (n = 2), *Chacophrys pierottii* (n = 9), *Lepidobatrachus asper* (n = 2), *Lepidobatrachus laevis* (n = 1) (Appendix F). Items in the stomach and intestine were counted and identified to the lowest taxonomic resolution possible. I grouped the food items into generalized categories and calculated the percent frequency of occurrence for each species. Because most of the prey items were incomplete, and I lacked a reference

collection for sizes of prey items, I did not calculate their percent volume in the gut of the specimens.

Field observations

I report five new prey items for ceratophryid frogs through direct observations in the field. Three new prey items are documented for larval and post-metamorphic *Ceratophrys cranwelli* gathered from opportunistic encounters (C.M. Schalk, pers. obs.) in the dry Chaco forest of southeast Bolivia around the community of Yapiroa, Provincia Cordillera, Departmento de Santa Cruz, Bolivia (19°36’15’’S, 62°34’32’’W). The dry Chaco is a xerophytic thorn forest with a distinct rainy (November–March) and dry (October–November) season, averaging 513 mm of rainfall annually in this region (Navarro and Maldonado, 2002). Another new prey item is documented from an opportunistic encounter (E.R. Wild, pers. comm.) in the diet of adult *Ceratophrys cornuta* from Reserva Amazonica, Peru. Reserva Amazonica is a tourist lodge and reserve on the north bank of the Río Madre de Dios, about 15 km ENE of Puerto Maldonado, Provincia de Tambopata, Departamento de Río Madre de Dios, Peru (12°33’ S, 69°03’ W; 200 m) (Duellman and Koechlin, 1991). The climate is seasonally tropical with distinct rainy (October–March) and dry (May–July) seasons and an average annual rainfall of 2416 mm. The region is mapped as a humid tropical forest, but is situated near the transition between humid tropical forest and dry tropical forest (Tosi, 1960). Another new prey item is documented in the diet of *Lepidobatrachus laevis* from field observations at Laguna Yema Formosa, Argentina (J. Faivovich, pers. comm.).

Results

The literature survey found 42 references on the diet of eight of the twelve species of Ceratophryidae from captive and field studies (Tables 4 and 5, respectively); this total does not include citations of previous reports. Those species for which no data could be found in the literature are *Ceratophrys calcarata*, *C. joazeirensis C. stolzmanni*, and *C. testudo*. Six of these eight ceratophryid species had information on tadpole diet, with sample sizes ranging from one to seven individuals. I provide novel observations regarding tadpole diet for only *C. cranwelli.* Regarding post-metamorphic diet I found literature information for all eight of these species, with sample sizes (again when reported) ranging from one to seventy-two individuals. The field observations and stomach content analyses provide additional post-metamorphic diet information (sample sizes ranging from one to nine) for all eight of these species except *Lepidobatrachus llanensis*, but with the addition of *C. calcarata*. Herein I provide a compilation of all these data, from both the literature and the observations (field and stomach content analysis), in the form of species accounts in which I first address tadpole diet, and then the diet of post-metamorphic life stage.

Ceratophrys aurita

In captivity, the tadpoles of *Ceratophrys aurita* have been observed consuming three species of tadpoles, (Miranda-Ribeiro, 1923; Table 4). Noble (1927) commented that tadpoles of *C. aurita* are cannibalistic. He also dissected a single tadpole of *C. aurita* revealing the presence of tadpoles and ostracods in its diet (Noble, 1927; Table 4). Izecksohn and Carvalho-e-Silva (2001) claim that this species breeds in newly formed

puddles along with other explosive breeders, especially microhylids, whose tadpoles serve as food for tadpoles of *C. aurita*.

One species of anuran was confirmed in the diet of post-metamorphic *C. aurita*, the microhylid *Hyophryne histrio* (Targino and Wild, 2009). Although there has been a report of an additional anuran species, *Trachycephalus* cf. *mesophaeus*, it cannot be confirmed (see Solé et al., 2010). To this, I add that seven individuals of *Chiasmocleis* sp. (SVL 18.5–9.0 mm) were found (along with the single *Hyophryne histrio* of Targino and Wild, 2009) in the stomach of a single juvenile specimen (SVL = 55.3 mm) from Nova Viçosa municipality (17°52’ S, 39°23’ W) in the southern part of the State of Bahia, Brazil (MNRJ 19030). Dissection of this, and two other individuals (MNRJ 19031, KU 92740) revealed that arthropods, especially ants of the family Formicidae were the most commonly encountered prey (Tables 6 and 7). Izecksohn and Carvalho-e-Silva (2001) report that the prey of *C. aurita* are usually vertebrates, such as amphibians, small birds or small mammals. Feio et al. (2008) mention the prey of *C. aurita* to be small vertebrates such as other amphibians, snakes, and rodents.

Ceratophrys calcarata

I did not find any information on the diet of the tadpoles of *Ceratophrys calcarata*.

Prior to this study, the only known prey item of *C. calcarata* was crickets fed to a captive individual while describing pedal luring behavior (Murphy, 1976). I found that arthropods, particularly coleopterans, contributed the largest proportion numerically to

the diet of *C. calcarata*, (n = 2; KU 144966, 207528) followed by vertebrates, which included an unidentified rodent and reptile (Tables 6 and 7).

Ceratophrys cornuta

Captive tadpoles of *Ceratophrys cornuta* were observed consuming five native species of tadpoles (Table 4; Duellman and Lizana, 1994), but nothing is known of their diet under natural conditions.

The study by Duellman and Lizana (1994) of *C. cornuta* in the Peruvian Amazon provides the most comprehensive and detailed study of the diet of any ceratophryid. In their examination of 72 stomachs, ants, and then beetles, had the greatest proportion numerically, while vertebrates, especially anurans and mammals, contributed the most volumetrically. They also examined prey frequency across a range of size classes, finding ants were again the most frequent prey item, but particularly for juveniles and adult males. This study also documented that every age class of this species consumes anurans. A range of prey items has been documented in the diet of *C. cornuta*, ranging from arthropods like orthopterans and hymenopterans to vertebrates such as lizards and mammals (Table 4). It has been reported that captive individuals in the field showed a clear preference for *Rhinella margartifera* over other offered native frogs species and non-rainforest cockroaches in the Peruvian Amazon (W. W. Lamar, pers. comm.). In addition to this, a medium-sized (ca. 50–90 g) oryzomyine rodent (*Euroryzomys* or *Hylaeamys*), was observed being eaten alive by an adult *C. cornuta* at Reserva Amazonica in the Peruvian Amazon (Figure 1a).

Ceratophrys cranwelli

The tadpole diet of *Ceratophrys cranwelli* is the best documented for any ceratophryid species. Cannibalism by *C. cranwelli* tadpoles has been reported by Gallardo and Varela de Olmedo (1992). Previous studies by Vera Candioti (2005; 2007) on seven tadpole specimens documented that Volvocaceae, insects, and crustaceans comprised the largest proportion of the diet of larval *C. cranwelli.* The tadpoles readily consumed tadpoles of *Pleurodema borelli* in captivity (Vera Candioti 2005; 2007), though I add that they consume tadpoles in the wild. On 23 March 2011 at 14:15 h, I observed a *C. cranwelli* tadpole that had recently captured a fairy shrimp (Crustacea: Anostraca) in shallow water along the edge of a temporary pond. The tadpole had captured the fairy shrimp head-first and proceeded to shear off the head and consume it. The tadpole then proceeded to shear off smaller bits from the body of the fairy shrimp until it had been completely consumed. The tadpole then swam off to a deeper part of the pond. The same day at 21:00 h while observing a small school of ten *Phyllomedusa sauvagii* (Hylidae) tadpoles grazing in a temporary pond along the water's surface, a *C. cranwelli* tadpole emerged from below and grasped the venter of an individual tadpole of *P. sauvagii*. The *C. cranwelli* tadpole shook the tadpole vigorously and proceeded to disembowel it, after which it swam to deeper water. The *C. cranwelli* did not return to consume the *P. sauvagii* tadpole that it had just attacked.

Both field observations and dissections have documented a wide variety of anurans and other vertebrates in the diet of post-metamorphic *C. cranwelli* (Table 4; Figure 1b). Here I add that on 23 March 2011 at 21:30 h I observed a metamorph *C.*

cranwelli (SVL = 30 mm) consuming a recently metamorphosed *P. sauvagii* along the edge of a temporary pond. The *P. sauvagii* still possessed a large tail-bud and had an orange-striped color pattern on its thighs and groin (Cei, 1980), suggesting that the individual had emerged from the nearby pond (approximately 20 cm away) that night. As in other observed predation events (Schalk, 2010; Schalk and Montaña, 2011), the prey item was still alive as it was being consumed, and made multiple attempts to force itself from the mouth of the *C. cranwelli*. During the brief periods when the *P. sauvagii* rested between escape attempts, the *C. cranwelli* used its hands and the ground to force the *P. sauvagii* further into its mouth. After 25 minutes, the *C. cranwelli* completely ingested the *P. sauvagii*. This prey species has been observed being rejected by other anurophagous frogs in the region (Scott and Aquino, 2005). The *C. cranwelli* was kept overnight to observe if there were any adverse effects of the *P. sauvagii* consumption on the *C. cranwelli*. The *P. sauvagii* was still retained by the *C. cranwelli* the following morning and the *C. cranwelli* was then released the following night.

These observations show the additional detail that *C. cranwelli* is anurophagous at all age classes, ranging from tadpoles to metamorphosed individuals (see observation above; Schalk, 2010) to adults (Schalk and Montaña, 2011). However, I cannot comment on the proportion of these prey items at various age classes, because these are based off of single observations. Nonetheless, in the two post-metamorphic individuals dissected, arthropods were the most common prey item, though we found for the first time evidence of this species having consumed a rodent (Tables 6 and 7). This species has

been reported to consume non-native prey items, consisting of both invertebrates and vertebrates, in captivity (Grayson et al., 2005).

Ceratophrys ornata

Other than several references commenting on their cannibalistic tendencies (Fernández and Fernández 1921; Noble 1927; 1931; Cei 1980), there is little other information on the diet of tadpoles of *Ceratophrys ornata* in nature (Table 4). In their laboratory study, Natale et al. (2011) offered as food tadpoles of seven different species of frogs (Table 5) known to share ponds with *C. ornata* in nature, all of which were consumed. These authors argued that the occurrence in nature of cannibalism by *C. ornata* larvae is likely rare because of low population densities of these tadpoles relative to the tadpoles of other synchronously reproducing frogs with high reproductive potential.

Gallardo (1974) fed captive adult *C. ornata* native orthopterans, mice, and two species of frogs including *C. ornata* (Table 5), but also non-native gastropods and beetles. Cei (1980) commented that adult *C. ornata* can consume small arthropods and small vertebrates, as did Gallardo (1987b), but did not provide any further details. In a feeding study over the course of 17 months, Braun et al. (1980) fed a single captive individual 17 species of anurans and two species of snakes (Table 4). Gallardo (1987a) and Gallardo and Varela de Olmedo (1992) report post-metamorphic individuals feeding on wide variety of vertebrates, plus arthropods and molluscs, and also reported cannibalism. The most complete examination of the natural diet of *C. ornata* is that of Basso (1990) who examined the contents of 34 stomachs and determined that anurans

were the most important of ten different prey categories found, both volumetrically (78.50%) and numerically (45.53%). Basso (1990) concludes that *C. ornata* is an anuran specialist, but noted that other vertebrates (birds, rodents, and a snake) and a variety of invertebrates (mostly coleopterans, isopods, and ants) were also consumed. Dissection of two specimens revealed that, numerically, vertebrates were the most frequent food items, though I did find both gastropod and arthropod invertebrates (Tables 6 and 7).

Chacophrys pierottii

As previously mentioned, tadpoles of *Chacophrys pierottii* lack the carnivorous traits of *Ceratophrys* and *Lepidobatrachus* and is a member of the benthic ecomorphological guild (Altig and McDiarmid, 1999; Quinzio et al., 2006). Polis and Myers (1985; Table 5, pg. 103) indicate in their table that the tadpoles of *C. pierottii* are cannibalistic citing Cei (1955) and Blair (1976); however, neither of these citations specifically mention tadpoles of *C. pierottii* being cannibalistic. Furthermore, Blair (1976) only makes the general assertion about "ceratophrynids" that "Both larvae and adults are carnivorous and cannibalistic" citing Cei (1955), Reig and Cei (1963), and "my data". I found no additional information on their diet in the wild.

Multiple authors have documented anurans in the diet of post-metamorphic *C. pierottii* (Vellard, 1948; Norman, 1994). In their study on the diet of juvenile *Chacophrys pierottii*, Pueta and Perotti (2013) revealed that hymenopterans and coleopterans were the dominant prey numerically and displayed the highest index of relative importance, whereas anurans and hymenopterans were the dominant prey volumetrically. Other prey items included scorpions, spiders, dipterans, and insect larvae

(Pueta and Perotti, 2013, Table 4). Cannibalism in juvenile *C. pierottii* was also documented (Pueta and Perotti, 2013). Besides the Pueta and Perotti (2013) study, there are only anecdotal reports that post-metamorphic individuals of this species are highly cannibalistic (Table 4). In the nine individuals dissected, arthropods comprised the majority of the diet, particularly larval and adult coleopterans, though I also found evidence of an unidentifiable vertebrate food item (Tables 6 and 7).

Lepidobatrachus asper

I did not find any information on the diet of *Lepidobatrachus asper* tadpoles.

All prior knowledge on the diet of post-metamorphic *L. asper* has been provided via anecdotes. Budgett (1899) commented that this species primarily feeds upon "*Bufo granulosus*" (=*Rhinella major*, Bufonidae). Other authors have documented cannibalism in this species and even more recent comments include frogs, arthropods, and gastropods as prey items (Table 4). In the four juvenile specimens dissected by Sugai et al. (2013), all the stomachs were empty but the intestines contained a few arthropods, plus plant material and sand particles which was considered to incidental to the ingestion of arthropod prey (Table 4). In the two specimens dissected, the only discernible items were larval coleopterans and detritus (Tables 6 and 7).

Lepidobatrachus laevis

Tadpoles of *Lepidobatrachus laevis* displayed cannibalism and were observed consuming non-native fish (*Poecilia* sp., *Carassius* sp.) in captivity (Ruibal and Thomas 1988, Table 5). Parker (1931) dissected a single wild caught individual to reveal that

tadpoles were the most frequently consumed prey, but also found that it consumed other small aquatic invertebrates (Table 4).

In the Paraguayan Chaco, at least six species of anurans have been found in the diet of post-metamorphic *L. laevis* (Table 4). Although feeding experiments by Scott and Aquino (2005) revealed that more species are palatable (Table 5), gastropods were the most frequent food item found in the stomachs. Other reported food items include arthropods, small vertebrates, and gastropods (Gallardo and Varela de Olmedo, 1992; Norman, 1994, Table 4). In the dissection of a single individual, I found arthropods as being the most frequent prey item (Tables 6 and 7). At Laguna Yema Formosa in Argentina, numerous *L. laevis* were collected that had their stomachs completely full with single individuals of freshwater snails of the genus *Pomacea* (J. Faivovich, pers. comm.). After leaving several adult individuals overnight in a sink, several opercula were found that were presumed to have been regurgitated. A few dissected specimens showed the snail shell to be perforated with numerous tiny holes, as if the gastric acids had started to dissolve the calcium carbonate of the shell (J. Faivovich, pers. comm.).

Lepidobatrachus llanensis

Aquatic invertebrates were the only food items found in the *Lepidobatrachus llanensis* tadpole specimens dissected by Cei (1968), while Vera Candioti (2007) found that tadpoles were the most frequent prey item, followed by crustaceans, and a negligible amount of diatoms in the four specimens she dissected (Table 5).

Dissection of 33 post-metamorphic individuals of *L. llanensis* from Argentina revealed that aquatic oligochaetes were the most common food item by both frequency

and volume (Hulse, 1978; Table 4). However, this species did consume a number of invertebrates and vertebrates, specifically anurans, such as *Leptodactylus bufonius*, *Pleurodema guayapae*, as well as its own species (Table 4).

Discussion

One general statement can certainly be said about the diet of frogs of the Ceratophryidae, in spite of their voracious feeding reputation much remains to be learned about what they eat during any of their life stages—in fact, I was unable to find any information at all for one-fourth of the species in the family (*Ceratophrys joazeirensis, C. stolzmanni,* and *C. testudo*). Many of the reports I did find on ceratophryid diet were anecdotal observations of only a few or individual specimens and often in captivity. Very few studies (Hulse, 1978; Basso, 1990; Duellman and Lizana, 1994; Pueta and Perotti, 2013) actually attempted to quantify the diet of naturally occurring ceratophryids. It seems the reputation of ceratophryid frogs as voracious megalophagous carnivores and cannibals is a generalization that has grown from very limited data. In fact, cannibalism by adults has only been reported for five of the twelve species, and by tadpoles for only four species. Yet I have found several authors that simply state, or at least imply, without data and often without a citation, that *all* ceratophryids are megalophagous and cannibalistic, and even when citations are given they concern only one or a few of the species. For example, the frequently referenced Blair (1976) states that "The ceratophrynids are much more cannibalistic than *Scaphiopus*. Both larvae and adults are carnivorous and cannibalistic (Cei, 1955b [Cei, 1955 of the present paper]; Reig and Cei, 1963; my data)." and "Adult *Chacophrys*

pierottii are extremely voracious cannibals; one of these can quickly ingest another individual of its own body size." However, "Cei 1955b" (herein cited as Cei 1955) only gives observations of cannibalism in adult *C. pierottii*, Reig and Cei (1963) only state that *L. asper* eats other frogs, insects, and will cannibalize, and Blair provides none of his "own data". Likewise, Fabrezi and Quinzio (2008) state "Adults of ceratophryines are megalophagous and are cannibals (Ruibal and Thomas, 1988; Hanken, 1993)." But Ruibal and Thomas (1988) only report cannibalism in *L. laevis* from captive individuals and state, without reference, "…*Ceratophrys* which has predatory carnivorous larvae." and Hanken (1993) only cites Ruibal and Thomas (1988) and gives no other source or data for his generalizations of feeding across all species of ceratophryids. So, herein I have attempted to distil the limited data that exists from the unsubstantiated generalizations, meanwhile adding additional information for eight species, including the first report on the diet of *C. calcarata*.

Tadpole diet

Of the twelve species of ceratophryids, tadpole diet data were known for only half (four species of *Ceratophrys* and two species of *Lepidobatrachus*). I added information for only one of these, *C. cranwelli*. The most commonly reported item in the tadpole diet of these species of *Ceratophrys* were tadpoles of other species within the families Bufonidae, Hylidae, Leptodactylidae, Microhylidae and Odontophrynidae. Two species, *C. aurita* and *C. ornata,* also have multiple reports of tadpole cannibalism in nature. Likewise, both of the species of *Lepidobatrachus* (*L. laevis* and *L. llanensis*), for which I found tadpole diet data, consumed tadpoles as well (reported as unidentified)

and *L. laevis* exhibited cannibalism, albeit in the laboratory (Ruibal and Thomas, 1988; E. R. Wild pers. observ.). Although there is no direct data regarding the diet of *C. pierottii* tadpoles, due to its morphology as a Type IV tadpole (Orton, 1953) of the benthic ecomorphological guild (Altig and McDiarmid, 1999), they are not expected to prey on tadpoles nor be cannibalistic, reports to the contrary notwithstanding (i.e., Polis and Myers, 1985).

It appears that tadpoles of the species of *Ceratophrys* and *Lepidobatrachus* prefer a tadpole diet, but whether they selectively choose various taxa or not is unclear. It is, however, noteworthy that *C. cranwelli* readily consumed, when offered, two species of bufonid tadpoles since these are generally considered toxic to vertebrates (Wassersug, 1971; Alford, 1999). Cannibalism among ceratophryid tadpoles is widely alleged, so it was surprising to find data limited to just five species, two of which remain questionable at best. The claim of cannibalism in *C. pierottii* is due to broad generalizations regarding the entire family that have been perpetuated (e.g., Blair, 1976; Reig and Cei, 1963; Polis and Myers, 1985) and remains in doubt. One of the most frequently cited papers for cannibalism in tadpoles of the Ceratophryidae is Reig and Cei (1963), however, they only report cannibalism by *L. laevis* based on captive individuals. For the other three species (*C. aurita*, *C. cranwelli*, and *C. ornata*), there is no conclusive evidence whether this cannibalism is intentional or that it is the incidental consumption of just another tadpole. Either way, this cannibalism serves as a strategy for accelerating development in the temporary aquatic habitats (Crump, 1983) that most ceratophryids utilize, and certainly does so for the three cannibalistic species identified here that reside in the Gran

Chaco. As a strategy for accelerating development, cannibalism assures an abundant local food source, it eliminates competitors, and provides additional thyroxin, all of which are known to contribute to more rapid development and metamorphosis (Pfennig, 1992; Wild, 1997b).

Since the diet of the tadpoles of *Ceratophrys cranwelli* is the best documented for any ceratophryid species, these findings for this species may be particularly enlightening to the nature of all *Ceratophrys* tadpoles. These observations provide further evidence as to the predatory nature of tadpoles of *C. cranwelli*. While Vera Candioti (2005) noted that the tadpoles of *C. cranwelli* often engulfed tadpoles of *Pleurodema borelli* whole, the field observations document that these tadpoles will readily attack and consume prey species larger than themselves and appear to not be gape constrained. The additional observations I found documented in the literature also support the predatory nature of these tadpoles. While tadpoles of *Lepidobatrachus* are most likely to be obligate carnivores in the ponds which they inhabit, Vera Candioti (2005) studied the morphology and feeding mechanics of *C. cranwelli* and proposed that they may be facultative carnivores, exhibiting some degree of trophic variation. As many of the other species of *Ceratophrys* tadpoles are similar to *C. cranwelli* morphologically (e.g., Duellman and Lizana, 1994; Wild, 1997b), it is likely that these species are also facultative carnivores. Similarly, though the tadpole of *C. pierottii* is a member of the benthic ecomorphological guild, recent research of similar tadpole ecomorphs has suggested that they may be omnivores or even carnivores (Altig et al., 2007).

Knowledge of the functional role of the ceratophryid tadpole is based on extremely small sample sizes, with the largest being seven individuals, and often limited to individuals collected from a single pond. Furthermore, the resources that these tadpoles utilize are likely to vary across both pond type and season (C.M. Schalk, unpubl. data). Specimen dissections are time-consuming and may provide only a snapshot of the prey items in the gut of these species, so stable isotopes may be a more feasible alternative to understand the functional role of these species (*sensu* Altig et al., 2007), in particular they may be useful for those species that may be inherently plastic in their trophic ecology (i.e., *C. pierottii* and *Ceratophrys* spp.).

Post-metamorphic diet

Prior to this study, adult diet data were known for eight species of ceratophryids; I was unable to find any previous information on the post-metamorphic diets of *C. calcarata*, *C. joazeirensis*, *C. stolzmanni*, and *C. testudo.* However, I do add here, for the first time, data on the diet of post-metamorphic *C. calcarata*. All these species have been documented to eat vertebrates in nature and all, except *C. calcarata*, consumed frogs (primarily of the families Bufonidae, Hylidae, Leptodactylidae, and Microhylidae, but there are also instances of Craugastoridae and Odontophrynidae). However, the lack of evidence of anurophagy in *C. calcarata* may simply be due to the small sample size. One emergent pattern I observed is that prey items of the other major vertebrate groups (Reptilia, Mammalia, Aves, Actinopterygii) were only found among species of the genus *Ceratophrys*, with each species having prey from at least two of these groups (most often Reptilia and Mammalia; Table 5). On the other hand, none of the species of

Lepidobatrachus or *C. pierottii* had any prey items from among these non-anuran vertebrate groups. This may be because of the terrestrial versus aquatic nature of *Ceratophrys* and *Lepidobatrachus,* respectively, yet it does not explain the absence of non-anuran vertebrates in *Chacophrys* (terrestrial) or the only fish item being found in *C. ornata* (terrestrial). The most comprehensive study of the diet of *C. pierottii* only focused on post-metamorphic juveniles (Pueta and Perotti, 2013), which may experience constraints imposed by their limited gape size (as compared to adults) and are unable to consume larger vertebrate prey. Furthermore, cannibalism by adults was documented in only one species of *Ceratophrys* (*C. ornata*) but observed in all species of *Lepidobatrachus* and *C. pierottii*. Adults of one species (*L. llanensis*) had tadpoles in its diet, which is not a surprise because of their aquatic nature.

Species of ceratophryids are by no means limited to vertebrate prey, and I realize that this compilation is likely to be biased to the larger, more easily observable prey items. In fact, all of the observations from feeding experiments or captive individuals used only vertebrates as prey, which likely perpetuated the idea that these species primarily feed upon vertebrates. However, the few thorough and detailed studies of post-metamorphic diet of these species (i.e., *L. llanensis* by Hulse, 1978; *C. ornata* by Basso, 1990; *C. cornuta* by Duellman and Lizana, 1994; *C. pierottii* by Pueta and Perotti, 2013) show the extremely broad spectrum of items in the diet of these frogs, ranging from invertebrates to vertebrates. For many of the other ceratophryid species, the largest proportion of the information available on their diet come from laboratory feeding studies. While these laboratory studies address the question of palatability of potential

prey species, they do not provide information of which species contribute an important portion of their diet under natural conditions.

Generalities that can be made; all species of *Ceratophrys* that have been studied for diet appear to be generalist, opportunistic feeders such as was demonstrated for *C. cornuta* (Duellman and Lizana, 1994). But there is yet to be any study on prey availability in their natural habitats, which is needed to confirm this suspicion. Certainly there are many more small prey items in their diet than their reputation suggests, and although they do consume larger prey, including vertebrates nearly of their own size, these seem to be opportunistic encounters. For those species without data, phylogenetically they would be expected to have similar diets.

This review also highlights some notable observations that deserve comment. The first being the observation of pedal luring behavior by adult *C. aurita* (Izecksohn and Carvalho-e-Silva, 2001), which is only the third report of this behavior among ceratophryids, the other species being *C. calcarata* (Murphy, 1976) and *C. ornata* (Radcliffe et al., 1986). This may be more widespread but perhaps phylogenetically limited to the genus *Ceratophrys*. The second observation that deserves comment is the multiple on the aggressive nature of these species during reproductive bouts. Female *C. cranwelli* have been observed biting a male conspecific during multiple mating attempts (Silva et al., 2014), and even after being amplexed, female *C. cranwelli* have been observed consuming or attempting to capture heterospecific anurans (see photograph in Schalk and Montaña, 2011, and report by Silva et al. 2014). Whether aggressive interactions between males and females are more widespread in other species of

ceratophryids, and whether predation of males by females actually occurs is unknown, but deserve further investigation. The last observation that deserves comment is the evidence of durophagy (consumption of hard-bodied food items) via the consumption of large freshwater snails by post-metamorphic *Lepidobatrachus laevis* (J. Faivovich pers. comm.; Scott and Aquino, 2005). Whether snails are a regular part of *L. laevis* diet, or if other species of *Lepidobatrachus* consume snails as well, the digestive abilities of *L. laevis* and the nutritional value of these prey are interesting and warrants further investigation.

Changes during ontogeny

The ontogenetic niche shift associated with amphibian metamorphosis is a well-studied ecological phenomenon (Wilbur, 1980; Werner and Gilliam, 1984), but fewer studies have examined the niche shifts occurring in the post-metamorphic life stage from metamorph to adult. Whereas there is a fair amount known about the ontogenetic changes in morphology associated with feeding in ceratophryids (Fabrezi and Emerson, 2003; Wild, 1997a, 1997b), these have yet to be correlated with changes in diet. Body size is an important factor influencing a species' trophic ecology, such as morphological constraints on prey size, as well as its vulnerability to predators, competitors, and physiological constraints which can influence the selection of ambush sites. Some ceratophryids can undergo over a four-fold increase in body size during post-metamorphic ontogeny (Wild, 1997b), and therefore one would expect ontogenetic niche shifts to be pervasive in these species. For example, in the Bolivian Chaco, *C. cranwelli* exhibited an ontogenetic shift in ambush sites with metamorphs and adults selecting

different sites (Schalk & Fitzgerald 2015). Even after this review and addition of observations, comments about the ontogenetic changes in diet remain speculative because so few studies have diet data available across multiple size classes and with an adequate sample size. Future efforts should attempt to examine the diet across different size classes.

Conclusions

In spite of the voracious feeding behavior and the many generalizations of megalophagy and cannibalism attributed to frogs of the Ceratophryidae, the few thorough studies on diet in nature suggest that most are likely generalist, opportunistic predators and that a clear understanding of their diet has yet to be achieved. Certainly most ceratophryids are capable of eating relatively large food items, but many other items are indeed found in their diet. Questions remain such as what feeding strategy do these frogs employ in choosing prey? Are they indeed choosing large items or are they sampling preying opportunistically on anything that they encounter? Similar questions can be posed regarding the larval stage as well as even less information is known about the diet of the tadpoles of these species in their breeding ponds. These species are cryptic and thus difficult to locate in the environment, making collecting and opportunistic encounters difficult. Future efforts should attempt to document the diet of all life stages of these frogs, especially for those species for which data are completely lacking.

Table 4. A compilation of the known prey of the frog species in the family Ceratophryidae compiled from field observations or specimen dissections (including the specimens dissected for this study). Life stage abbreviations are T = tadpole and PM = post-metamorphosis. All species of anuran prey are post-metamorphic frogs unless otherwise specified. Species names follow Frost (2014).

Species	Life Stage	Prey	Reference
C. aurita	T	*Ceratophrys aurita* tadpoles *Hyla* sp. tadpole Unidentified tadpoles Ostracoda	Noble, 1927
	PM	*Hyophryne histrio*	Targino and Wild, 2009
		Trachycephalus cf. *mesophaeus*	Solé et al., 2010
		Chiasmocleis sp. Acarina	This study
		Formicidae Hymenoptera Ixodidae Orthoptera Unidentified reptile Unidentified vertebrate Unidentified anuran Unidentified arthropod	

Table 4. Continued.

Species	Life Stage	Prey	Reference
		Amphibians Small birds Small mammals	Izecksohn and Carvalho-e-Silva, 2001
		Amphibians Snakes Rodents	Feio et al., 2008
C. calcarata	PM	Coleoptera adult Formicidae Unidentified reptile Unidentified arthropod Unidentified rodent	This study
C. cornuta	PM	Orthoptera Araneae *Edalorhina perezi*	Duellman, 1978
		Leptodactylus dydimus *Plica plica*	Chávez et al., 2011
		Oligochaeta Gastropoda Crustacea Aranea Acarina Miriapoda Orthoptera Homoptera Heteroptera	Duellman and Lizana, 1994

Table 4. Continued.

Species	Life Stage	Prey	Reference
		Diptera	
		Diptera larvae	
		Formicidae	
		Hymenoptera	
		Coleoptera adult	
		Coleoptera larva	
		Unidentified arthropod	
		Rhinella margaritifera	
		Pristimantis toftae	
		Hamptophryne boliviana	
		Dendropsophus parviceps	
		Scinax ruber	
		Hypsiboas punctatus	
		Ptychoglossus brevifrontalis	
		Anolis fuscoauratus egg	
		Unidentified snake	
		Oecomys bicolor	
		Unidentified mice	
		Oryzomyine rodent	This study
C. cranwelli		*Phyllomedusa sauvagii*	
		tadpoles	This study
		Anostraca	
	T	Volvocaceae	
		Crustacea	Vera Candioti, 2005
		Desmidiaceae	
		Euglenoids	

Table 4. Continued.

Species	Life Stage	Prey	Reference
		Insecta Nematoda	
		Oligochaeates Rotifers Shell-bearing amoebas Vegetal remants Diatoms	
		Macrophytes Tecamebians	Vera Candioti, 2007
		Ceratophrys cranwelli tadpoles	Gallardo and Varela de Olmedo, 1992
	PM	Anurans Small mammals Birds	Contreras and Contreras, 1982
		Lizards Mollusks	
		Anurans	Norman, 1994
		Dermatonotus muelleri	Wild, 2001
		Leptodactylus bufonius	Schalk, 2010
		Physalaemus albonotatus	Schalk and Montaña, 2011
		Physalaemus biligonigerus	Wild, 2001; Scott and Aquino, 2005
		Leptodactylus sp. *Rhinella major* Coleoptera adult	Scott and Aquino, 2005

Table 4. Continued.

Species	Life Stage	Prey	Reference
		Phyllomedusa sauvagii Unidentified arthropod Formicidae Coleoptera adult Unidentified rodent	This study
C. ornata		*Ceratophrys ornata* tadpoles	Noble, 1927, 1931; Cei, 1980
	T	Tadpoles Small crustaceans	Gallardo, 1987a; Gallardo and Varela de Olmedo, 1992
	PM	Small vertebrates Arthropods	Cei, 1980; Gallardo, 1987b
		Ceratophrys ornata Amphibians Lizards	Gallardo, 1987a; Gallardo and Varela de Olmedo, 1992
		Snakes Juvenile birds Rodents Arthropods Gastropoda-slugs and snails	
		Amphisbaena darwini	Gallardo and Varela de Olmedo, 1992
		Coleoptera Araneae Isopoda	Basso, 1990

Table 4. Continued.

Species	Life Stage	Prey	Reference
		Formicidae Gastropoda Hemiptera	
	PM	Rodents Snakes Birds Frogs	Basso, 1990
		Gastropoda Unidentified anuran Unidentified arthropod Unidentified fish Unidentified rodent	This study
Ch. pierottii		*Physalaemus* spp. *Leptodactylus* spp. juveniles	Vellard, 1948
		Chacophrys pierottii	Cei, 1955, 1980; Blair, 1976;
		Anurans	Norman, 1994
		Apidae Formicidae Unidentified Hymenoptera Curculionidae Dytiscidae Heteroceridae Elateridae	Pueta and Perotti, 2013

Table 4. Continued.

Species	Life Stage	Prey	Reference
		Tenebrionidae Coccinelidae Pentatomidae Unidentified Hemiptera Diptera adult Diptera larvae	
		Araneae Scorpionidae Lepidoptera larvae Unidentified insect larvae Unidentified anuran *Chacophrys pierottii*	
		Coleoptera adult Coleoptera larva Hemiptera Insect larva Unidentified vertebrate Unidentified arthropod	This study
L. asper		*Rhinella major*	Budgett, 1899; Gadow, 1909
		Frogs Insects	Reig and Cei, 1963
	PM	*Lepidobatrachus asper*	Cei, 1958; Reig and Cei, 1963; Cochran, 1961
		Arthropods Small vertebrates	Cei, 1980; Gallardo, 1987b; Gallardo and Varela de Olmedo, 1992

Table 4. Continued.

Species	Life Stage	Prey	Reference
		Coleoptera adult	Reig and Cei, 1963; Cei, 1980; This study
		Gastropoda Anurans	Norman, 1994
		Aquatic Coleoptera Terrestrial Coleoptera Araneae Acari Blattodea	Sugai et al., 2013
L. laevis	T	Unidentified tadpoles Clam shrimp Coleoptera Unidentified aquatic insect	Parker, 1931
	PM	Athropods Small vertebrates	Gallardo and Varela de Olmedo, 1992
		Gastropoda Anurans	Norman, 1994
		Rhinella major *Pseudis paradoxa* *Dermatonotus muelleri* *Physalaemus biligonigerus* *Physalaemus albonotatus* *Leptodactylus* spp. Unidentified anuran Arthropoda Gastropoda	Scott and Aquino, 2005

Table 4. Continued.

Species	Life Stage	Prey	Reference
		Gastropoda (*Pomacea*)	Faivovich pers. comm.
		Coleoptera adult Unidentified arthropod	This study
L. llanensis		Hemiptera larvae Notostraca	Cei, 1968
	T	Diatoms Crustaceans Unidentified tadpoles	Vera Candioti, 2007
	PM	Oligocheates Eubranchipoda Unidentified tadpoles Unidentified anurans Carabidae Belostomatidae nymphs Hymenoptera *Lepidobatrachus llanensis*	Hulse, 1978
		Pleurodema guayapae Lepidoptera Coleoptera Gastropoda Anisoptera nymphs *Leptodactylus bufonius* Notonectidae Scarabidae Araneae	Gallardo and Varela de Olmedo, 1992

Table 4. Continued.

Species	Life Stage	Prey	Reference
		Diptera Arthropods Small vertebrates	Gallardo and Varela de Olmedo, 1992

Table 5. A compilation of the known prey of the frog species in the family Ceratophryidae consumed while in captivity or as part of a feeding experiment using native potential prey. Studies conducted with non-native potential prey are not included in this table. Life stage abbreviations are T = tadpole and PM = post-metamorphosis. All species of anuran prey are post-metamorphic frogs unless otherwise specified. Species names follow Frost (2014).

Species	Life Stage	Prey	Reference
C. aurita	T	*Ceratophrys aurita* tadpoles *Leptodactylus* sp. tadpoles *Leptodactylus latrans* tadpoles	Miranda-Ribeiro, 1923
	PM	Young chickens	Miranda-Ribeiro, 1920
C. cornuta	T	*Dendropsophus koechlini* tadpoles *Ctenophryne geayi* tadpoles *Chiasmocleis ventrimaculata* tadpoles	Duellman and Lizana, 1994
		Dendropsophus leucophyllatus tadpoles *Elachistocleis ovalis* tadpoles	
	PM	*Rhinella margaritifera*	W. W. Lamar pers. comm.
C. cranwelli	T	*Pleurodema borelli* tadpoles	Vera Candioti, 2005, 2007
C. ornata	T	*Hypsiboas pulchellus* tadpoles *Scinax squalirostris* tadpoles	Natale et al., 2011

Table 5. Continued.

Species	Life Stage	Prey	Reference
		Odontophrynus americanus tadpoles *Leptodactylus latrans* tadpoles *Rhinella arenarum* tadpoles	
		Rhinella fernandezae tadpoles	Natale et al., 2011
		Ceratophrys ornata tadpoles	Fernández and Fernández, 1921
	PM	Frogs *Ceratophrys ornata*	Gadow, 1909
		Orthoptera *Ceratophrys ornata* *Rhinella arenarum*	Gallardo, 1974
		Mice	
		Dendropsophus minutus *Dendropsophus sanborni* *Hypsiboas leptolineatus*	Braun et al., 1980
		Hypsiboas pulchellus *Leptodactylus gracilis* *Leptodactylus latinasus* *Leptodactylus latrans* *Odontophrynus americanus* *Physalaemus gracilis* *Pseudis minuta* *Rhinella arenarum* *Rhinella crucifer*	

Table 5. Continued.

Species	Life Stage	Prey	Reference
		Rhinella dorbignyi *Rhinella fernandezae* *Scinax fuscovarius* *Scinax squalirostris*	
		Unidentified hylid *Liophis jaegeri* *Liophis poecilogyrus*	
L. laevis	T	*Lepidobatrachus laevis* tadpoles	Ruibal and Thomas, 1988
	PM	*Rhinella schneideri* *Lepidobatrachus laevis* *Leptodactylus bufonius* *Leptodactylus chaquensis* *Leptodactylus fuscus* *Phyllomedusa hypochondrialis* *Phyllomedusa sauvagii*	Scott and Aquino, 2005

Table 6. Number of food items found from dissections in seven species of frogs of the family Ceratophryidae. MW = mouth width.

	Ceratophrys				*Chacophrys*	*Lepidobatrachus*	
	aurita	*calcarata*	*cranwelli*	*ornata*	*pierottii*	*asper*	*laevis*
Number of Stomachs	3	2	2	2	9	2	1
SVL Range (mm)	47.5–104.5	62.2–62.6	53.9–71.5	52.3–79.6	29.1–38.3	43.9–56.6	46.2
MW Range (mm)	29.8–68.6	38.5–43.1	29.6–48.8	36.446.9	17.0–19.9	29.4–33.6	30
Acarina	2						
Chiasmocleis sp.	8						
Coleoptera adult		10	3		13	2	2
Coleoptera larva					16		
Detritus					2	1	1
Formicidae	32	2	1		2		
Gastropoda				1			
Hyophryne sp.	1						
Hemiptera					1		
Hymenoptera	2						
Insect larva					1		

Table 6. Continued.

	Ceratophrys				*Chacophrys*	*Lepidobatrachus*	
	aurita	*calcarata*	*cranwelli*	*ornata*	*pierottii*	*asper*	*laevis*
Isoptera	2						
Ixodidae	1						
Lepidoptera larva					5		
Orthoptera	2						
Plant matter	1	1	1	1	4		1
Stone							1
Unidentified reptile	1	1		1			
Unidentified vertebrate	1				4		
Unidentified anuran	1			1			
Unidentified arthropod	2	2	2	1	8		1
Unidentified fish				1			
Unidentified rodent		1	2	1			
Unknown	2		1	1	4		
Total	58	17	10	8	60	3	6

Table 7. Numerical proportions of general prey categories found from dissections in seven species of frogs of the family Ceratophryidae. MW = mouth width.

	Ceratophrys				*Chacophrys*	*Lepidobatrachus*	
	aurita	*calcareta*	*cranwelli*	*ornata*	*pierottii*	*asper*	*laevis*
Number of Stomachs	3	2	2	2	9	2	1
SVL Range (mm)	47.5–104.5	62.2–62.6	53.9–71.5	52.3–79.6	29.1–38.3	43.9–56.6	46.2
MW Range (mm)	29.8–68.6	38.5–43.1	29.6–48.8	36.4–46.9	17.0–19.9	29.4–33.6	30
Arthropods (%)	74.1	82.4	60.0	12.5	76.7	66.7	50.0
Gastropods (%)	0.0	0.0	0.0	12.5	0.0	0.0	0.0
Vertebrates (%)	20.7	11.8	20.0	50.0	6.7	0.0	0.0
Other (%)	5.2	5.9	10.0	25.0	16.7	33.3	50.0

Table 8. Major groups of vertebrates (left) and amphibians (right) as prey of post-metamorphic individuals of species of the Ceratophryidae. All instances are observations in nature except those indicated by a boldface **X** which were in captivity of part of a feeding experiment using native potential prey.

Predator Species of the Ceratophryidae	Major vertebrate groups as prey					Amphibian groups as prey							
	Amphibia	Reptilia	Mammalia	Aves	Actinopterygii	Tadpoles	Cannibalism	Microhylidae	Hylidae	Leptodactylidae	Bufonidae	Craugastoridae	Odontophrynida
Ceratophrys													
aurita	X	X	X	X				X	X				
calcarata		X	X										
cornuta	X	X	X					X	X	X	X	X	
cranwelli	X	X	X	X				X	X	X	X		
ornata	X	X	X	X	X		X		**X**	**X**	**X**		**X**
Chacophrys													
pierottii	X						X			X			
Lepidobatrachus													
asper	X						X						
laevis	X						**X**	X	X	X	X		
llanensis	X					X	X			X			

CHAPTER VIII

SUMMARY AND CONCLUSIONS

Globally, the Gran Chaco ecoregion is experiencing one of the highest deforestation rates in the world making it one of the most threatened ecosystems in the world (Zak et al. 2004). As nearly one-third of amphibians are experiencing population declines, design of conservation efforts are further complicated with the fact that most anuran amphibians possess a complex life-cycle and undergo an ontogenetic niche shift where an aquatic larvae metamorphoses to a terrestrial adult (Wilbur 1980). Thus conservation plans must be appropriately designed to target conservation needs for both life stages. The series of studies and experiments I carried out to complete my dissertation was to understand the factors that are influencing community assembly patterns of Neotropical amphibians across multiple spatial and temporal scales across both the larval and post-metamorphic life-stages. Results from this dissertation can be applied to design and implement conservation efforts in the Gran Chaco ecoregion and the broader tropics as a whole.

Amphibian diversity is higher in the tropics than anywhere else on earth (Duellman and Trueb 1986). Not only is species diversity higher in the tropics, but the functional and morphological diversity of these species is greater than compared to regions at higher latitudes. My research showed while building on a growing body of literature, that the process of environmental filtering plays a strong role in driving the community structure of anurans in the Gran Chaco ecoregion. An overarching finding

was that co-occurring species in anuran assemblages responded to strong environmental gradients and this appeared to determine community structure (Chapter II).

I can only speculate about long-term implications for conservation of Chacoan amphibian communities. For example, global warming has been put forth as a conservation challenge for the persistence of arid-adapted frogs. Under scenarios of increasing temperature due to climate change, the arid-adapted anurans of the Gran Chaco may be susceptible to warming-induced extinctions (Duarte et al. 2012). Although amphibians can adapt to arid environments by either becoming more fossorial or increasing their association with more permanent aquatic habitats (Blair 1976), there are few permanent aquatic habitats available to frogs in the Chaco. Most frog species inhabiting this highly seasonal and unpredictable environment breed in temporary ponds. Moreover, my results suggest that these ephemeral pond breeding species lack the functional traits associated with a fossorial lifestyle, and therefore may be more prone to warming induced extinctions.

Amphibians exhibit large natural fluctuations in their population dynamics at both the larval and post-metamorphic life stages (Pechmann et al. 1991; Semlitch et al. 1996). Conservationists must recognize that many aspects of amphibian ecology are plastic, including resource use and response to predators. For example, while predation and pond drying are important factors that structure tadpole communities (Heyer et al. 1975; Skelly 1996), but it appears that many larval anurans more generalized in regards to their trophic ecology and capable of exploiting diverse resources within the breeding ponds (Chapter III). Furthermore, their trophic ecology appears to be independent of

their ecomorphological guild classification (Chapter III). This generalized feeding behavior presents a challenge to understanding their functional role within aquatic habitats, especially if one wants to know the consequences of losing these abundant consumers from aquatic ecosystems (Whiles et al. 2006; Altig et al. 2007). Future efforts should attempt to determine if additional context-dependent factors (e.g., presence of predators, competitors) that may influence tadpole trophic ecology in an attempt to develop a robust predictive framework at their functional role.

The potential problem that climate change poses to amphibians is not necessarily increasing temperatures per se. It is important to note that amphibians often exhibit the strongest response to climate change in the form in advancements or delays in onset and duration of breeding (Parmesan 2007). These delays or advancements in breeding phenology have the potential to result in mistimed interspecific interactions or reproductive events at the subsequent larval life-stage (Beebee 1995; Saenz et al. 2006; Todd et al. 2010). My results suggest that the breeding phenology of tropical amphibians is the product of endogenous (e.g., reproductive modes) and exogenous (e.g., weather) factors (Chapter IV). Even subtle changes in weather and climatic patterns can affect the reproductive activity of Chacoan anurans, which could carry over and affect interactions at the next life stage.

Complex oviposition strategies are believed to have evolved as a means to reduce exposure of eggs and larvae to predators by reducing the amount of time spent in aquatic habitats, or to protect eggs from desiccation (Magnusson & Hero 1991; Crump 2015). While previous studies have recognized the timing of breeding for frogs can carry over

to affect interspecific and intraspecific interactions occurring at the larval life stage (Saenz et al. 2006; Chapter V), the effect of a species' reproductive mode on the larval life stage and the role they have in influencing community structure is not well known. My results suggest that terrestrial reproductive modes may decrease constraints imposed by the ponds (specifically pond drying and exposure to predators) utilized as breeding habitats for certain species.

Habitat and diet are the most fundamental and important aspects of species' niches that biologists can easily study (Toft 1980; Pianka 2000; Morris 2003). These aspects of the ecological niche change during ontogeny of amphibians (Werner and Gilliam 1984). While the ontogenetic niche shift from aquatic larvae to terrestrial adult has been the subject of intensive study (Wilbur 1980; Werner 1986; Wells 2007), the niche shifts that occur within a single life stage have received far less attention. Results from this dissertation highlight that fundamental aspects of a species niche, such as its diet and habitat use, are lacking (Chapters VI and VII). It is difficult to detect small metamorphs of many species of anurans, and their ecology is not well known for many species (Wells 2007), but 90% of the growth occurs in the post-metamorphic life stage of some species (Werner 1986), suggesting considerable shifts in their niche. Conservation efforts need to recognize that that strengths and interactions of ecological factors vary during ontogeny when developing conservation strategies.

I couched my research efforts within the framework of Applied Biodiversity Science with the goal of conducting biodiversity research that was complementing the efforts of local actors and institutions. Specifically, I focused on building upon the

efforts of a community-based wildlife management program built between CABI and WCS (Noss et al. 2005) to expand the capacity of Isoceño parabiologists and park guards at the Kaa-Iya National Park. While originally trained in techniques associated with the monitoring terrestrial biodiversity, I sought to work with parabiologists to expand their capacity to train them to monitor and survey aquatic biodiversity. I worked together with parabiologists and park guards and trained them to identify the tadpoles, calls, and nests of the frogs of occurring in Isoso and the Kaa-Iya National Park. These efforts were highlighted by the organization of a workshop that complimented the efforts of the Kaa-Iya National Park to train parabiologists and park guards in these techniques. At the conclusion of this workshop, we developed a plan with the Kaa-Iya National Park director to begin monitoring aquatic habitats occurring at the various park guard posts across the park. Another local institution students from the Museo Noel Kempff Mercado to build their capacity and exposure to science added conservation value. I also worked with local Bolivian undergraduate students at the Museo Noel Kempff Mercado to train them in the biological sciences through the design and implementation of projects that served at their undergraduate thesis. These efforts focused on designing their capacity to measure and monitor biodiversity.

Working with local actors contributed to conservation capacity building and taught local people about science and frogs as a model system (O'Riordan & Stoll-Kleemann 2002). The multi-scale effort at understanding the patterns of frog community assembly will certainly help inform local conservation efforts. Even more important is the capacity of these local actors and institutions that can effectively design and

implement conservation strategies that include multiple aspects of biodiversity, both in the terrestrial and aquatic environments, as well as recognizing the need to consider the factors that structure biodiversity across different spatial and temporal scales as well as across different life stages. Moving forward, the stewardship built from working with these local institutions during the course of this dissertation will provide important momentum to conservation of the Bolivian Chaco.

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APPENDICES

Appendix A. Site by species list for the 39 frog species across 12 sites in the Gran Chaco ecoregion of Bolivia.

Family	Species	Tierras Nuevas	Yapiroa	Cerro Colorado	Cerro Cortado	Yande Yari	Cupesi	Tucavaca	La Madre	Salinas	Ravelo	Palmar	Cordabalan
Bufonidae	*Rhinella granulosa*	x	x	x	x	x	x	x	x	x		x	x
Bufonidae	*Rhinella schneideri*	x	x	x	x	x	x	x	x	x	x	x	x
Ceratophryidae	*Ceratophrys cranwelli*		x	x	x		x			x	x	x	
Ceratophryidae	*Chacophrys pierottii*					x							x
Ceratophryidae	*Lepidobatracus laevis*				x	x						x	x
Ceratophryidae	*Lepidobatracus llaenis*												x
Dendrobatidae	*Ameerega picta*							x					
Hylidae	*Dendrosophus melanargyreus*									x		x	
Hylidae	*Dendrosophus minutus*											x	
Hylidae	*Dendrosophus nanus*											x	
Hylidae	*Hysiboas raniceps*							x	x			x	

Appendix A. Continued.

Family	Species	Tierras	Yapiroa	Cerro	Cerro	Yande Yari	Cupesi	Tucavaca	La Madre	Salinas	Ravelo	Palmar	Cordabalan
Hylidae	*Phyllomedusa boliviana*										x		
Hylidae	*Phyllomedusa hypochondrialis*									x	x	x	x
Hylidae	*Phyllomedusa sauvagii*	x	x	x	x	x	x			x	x	x	x
Hylidae	*Pseudis paradoxa*												x
Hylidae	*Scinax acuminatus*												x
Hylidae	*Scinax fuscovarius*	x	x	x	x	x	x			x		x	
Hylidae	*Scinax nasicus*		x	x	x	x	x	x	x	x		x	x
Hylidae	*Trachycephalus typhonius*		x		x	x		x	x	x		x	
Leptodactylidae	*Leptodactylus bufonius*	x	x	x	x	x	x	x	x	x	x	x	x
Leptodactylidae	*Leptodactylus chaquensis*		x	x	x	x	x	x	x		x	x	x
Leptodactylidae	*Leptodactylus diptyx*					x	x	x	x				
Leptodactylidae	*Leptodactylus elenae*		x			x	x	x	x		x	x	
Leptodactylidae	*Leptodactylus fuscus*		x	x	x	x				x	x	x	x
Leptodactylidae	*Leptodactylus labyrinthicus*										x		
Leptodactylidae	*Leptodactylus laticeps*				x								x
Leptodactylidae	*Leptodactylus latinasus*		x					x	x				x
Leptodactylidae	*Leptodactylus leptodactyloides*					x	x		x				

Appendix A. Continued.

Family	Species	Tierras	Yapiroa	Cerro	Cerro	Yande Yari	Cupesi	Tucavaca	La Madre	Salinas	Ravelo	Palmar	Cordabalan
Leptodactylidae	*Leptodactylus mystacinus*	x								x	x	x	x
Leptodactylidae	*Physalaemus albonotatus*		x		x		x	x		x	x	x	x
Leptodactylidae	*Physalaemus nattereri*									x	x	x	
Leptodactylidae	*Physalaemus biligonigerus*	x	x	x	x	x	x	x		x	x	x	x
Leptodactylidae	*Pleurodema guayapae*		x										
Microhylidae	*Chiasmocleis albopunctata*							x			x		
Microhylidae	*Dermatonotus muelleri*	x	x	x		x	x	x	x	x	x	x	x
Microhylidae	*Elachistocleis bicolor*							x				x	x
Microhylidae	*Elachistocleis ovalis*			x	x	x		x				x	
Odontophrynidae	*Odontophrynus mericanus*		x		x					x	x	x	

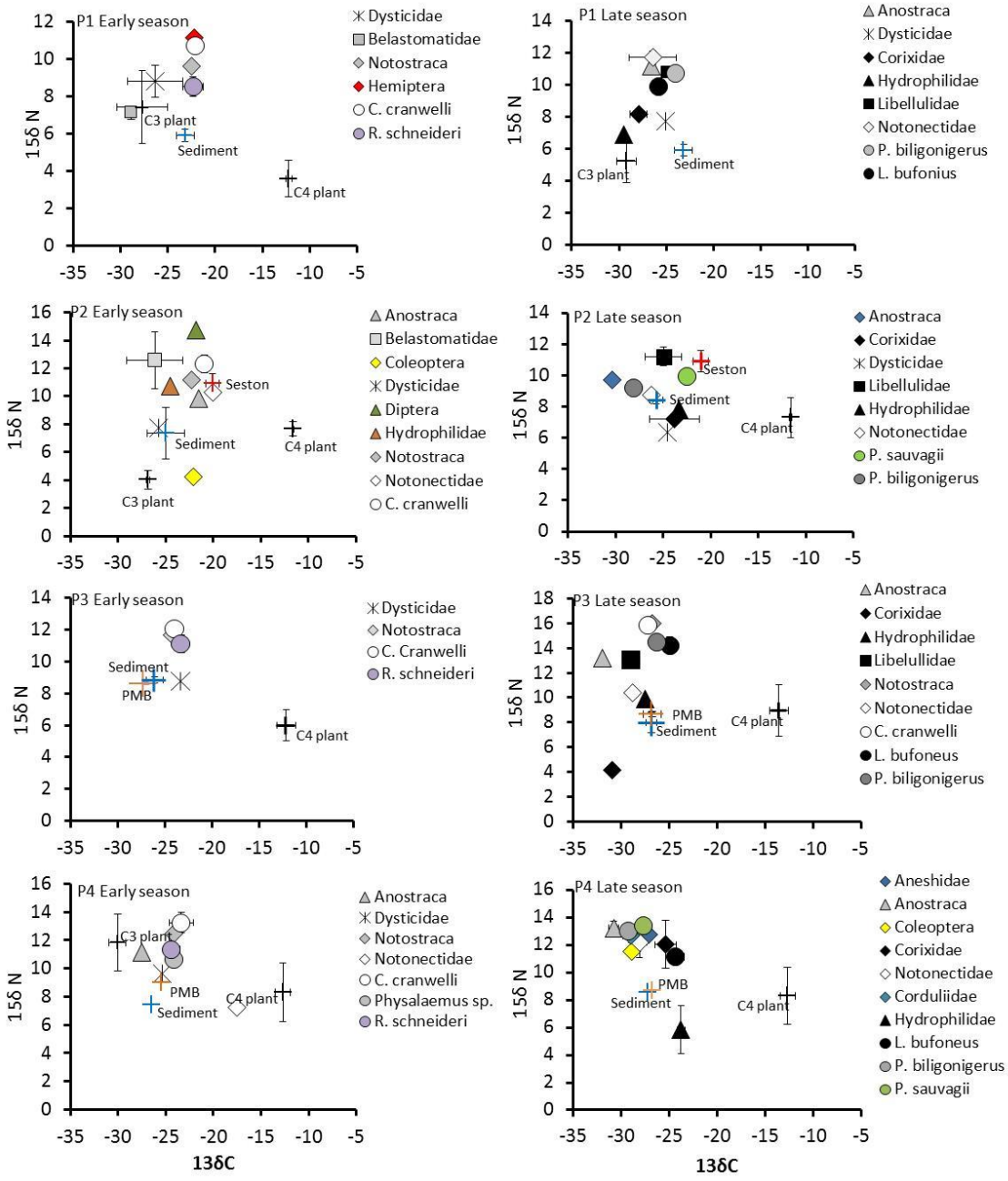
Appendix B. List of specimens measured from the Herpetology Collection at the Museo de Historia Natural Noel Kempff Mercado (MNKA) in Santa Cruz de la Sierra, Bolivia.

A. picta: 2848, 2849, 3209, 3212, 3211, 3210; *C. albopuncta*: 5901, 2702, 2258, 5908, 5744, 5743, 5837, 4505, 4495, 3418, 4543, 4546, 5682, 5680, 5681, 5920, 5891, 5897, 5934, 5910; C. cranwelli: 5633, 5683, 5653, 39, 41, 174, 4484, 4687, 556, 2992, 7738, 7739, 838, 7352, 7353, 7354, 4430, 10638; *C. pierottii*: 4234, 4235; *D. melanargyreus*: 5520, 5519, 545, 611, 414, 76, 409, 7560, 7518, 7556, 7562, 7543; *D. minutus*: 4398, 787, 790, 3015, 3165, 3166, 3164, 4531, 4532, 3500, 3483, 6446, 7909, 6354, 6754, 6753, 5518, 5517, 5516, 10218, 10222, 10219, 10220, 10221, 607, 603, 606; *D. nanus*: 8752, 835, 6580, 61, 5924, 5902, 203, 204, 8918, 5530, 5531, 5532, 5579, 5987, 3397, 3400, 3398, 3403, 3404, 3399, 3401, 812, 810, 815, 816, 811, 2853, 5557, 3502, 3261, 3292; *D. muelleri*: 4675, 4708, 5654, 5655, 5656, 5564, 5565, 4872, 4873, 7431, 7432, 7540, 4707, 5486, 2846, 6682; *E. bicolor*: 2300, 2688, 4781, 4783, 4617, 4616, 4618, 6204, 6205, 4619, 6166, 5805, 192, 3559, 2262, 2624, 2625, 4052, 5491, 5794, 153, 4041, 4757, 4758, 4756, 4920, 5001, 4042, 3470, 3468, 3469, 8508, 8481, 8480, 6225, 6223, 6224, 4554, 4075, 3413, 9195, 9199, 4799, 4805, 4804, 4806, 5679, 5672, 5676, 5675, 5677, 5678, 5670, 5673, 5674; *E. natterii*: 69, 70, 71, 8295, 7461, 5243, 6578; E. ovalis: 3032, 8917, 6374, 6384, 6395, 5542, 5543, 6410, 6683, 78, 3007, 1993, 1994, 1996, 3446, 4891, 4892, 2716, 2717, 1957, 1971, 6488, 4247, 2441, 5237, 5236, 3053, 8279, 6685, 6686, 4753, 4751, 4754, 4752, 4755, 4867, 4868, 4797, 4798, 4871, 4869, 4866, 4870, 4541, 4553, 4508, 4509, 5252; *H. raniceps*: 721, 3168, 8701, 2590, 4909, 14, 3173, 6243, 6244, 6241, 6242, 5774, 5775, 8486, 4522, 4520, 4523, 8606, 8604, 8603, 536, 341, 543, 537, 867, 8319, 4718, 3169, 1939, 1927, 1111, 6000, 6360, 6379, 6391, 6370, 6402, 638, 716, 676, 679, 1555, 1554, 1553, 4660, 4661, 4647, 1307, 1306, 3271, 3272, 3273, 3257, 3675, 3676, 2315, 2359, 2249, 2331, 2273, 2354, 2238, 2237, 8706, 8704, 8705, 8702, 8703, 2760, 5757, 2758, 2764, 2762, 2761, 2763, 2759, 2766, 2765; *L. bufonius*: 452, 399, 8526, 8507, 8484, 8483, 5645, 5644, 1370, 1365, 1365, 1366, 1363, 1367, 7537, 7571, 5560, 5558, 5559, 5614, 5613, 5612, 5615, 5239, 4646, 5907, 5694, 4656, 4655, 505, 1987; *L. chaquensis*: 4663, 5704, 5253, 5254, 5257, 3294, 10745, 170, 453, 2586, 3680, 2200, 2321, 8700, 8708, 8709, 2572, 2573, 2574, 2575, 2515, 2526, 2559, 2256, 820, 2862, 28, 1934, 1894, 1895, 1896, 1897, 1898, 1899, 2901, 2902, 2903, 2904, 2905, 2906, 2908, 1900, 1901, 1902, 1903, 1904, 1905, 1456, 2427, 2465, 2605, 1303, 1304, 1305, 5238, 2930, 2931, 2932, 2933, 2934, 2935, 5657, 5658, 5659, 1577, 1578, 1579, 1581, 3307, 3306, 3301, 3305, 3309, 3304, 3302, 3303, 3308, 4680, 4688, 298, 176, 2470, 2553, 2591, 2592, 2593, 177, 3208, 826, 828, 829, 830, 831, 836, 1452, 1453, 4818, 5562, 4801, 4803, 4923, 5478, 2223, 2224, 2257, 2479, 2595, 2691, 2692, 3432, 2694, 2698, 2620, 2629, 2705, 2690, 2693, 2710, 2689, 2632, 2616, 2709, 2714, 2713, 1760, 5477, 1898, 2306, 2484, 1949, 1959, 2603, 266; *L. diptyx*: 3706, 5948, 4748, 4691, 5887, 4840, 2707, 2635, 8476, 8506, 8492, 8495, 8475, 4493, 4494, 4535, 5484, 5483, 5482, 5789, 5888, 2302, 2578, 4743, 4749, 4739, 4741, 4750, 4740, 4742, 4745, 4744, 4747, 4746, 4738; *L. elenae*: 837, 5636, 5637, 4496, 5862, 318, 9242, 5651, 6236, 1936, 4762, 4764, 3062, 3070, 4993, 8518, 8521, 4986, 4987, 5859, 9204, 5497, 398, 5240, 4989, 5861, 2929, 2856, 2860, 2857, 2859, 2858,

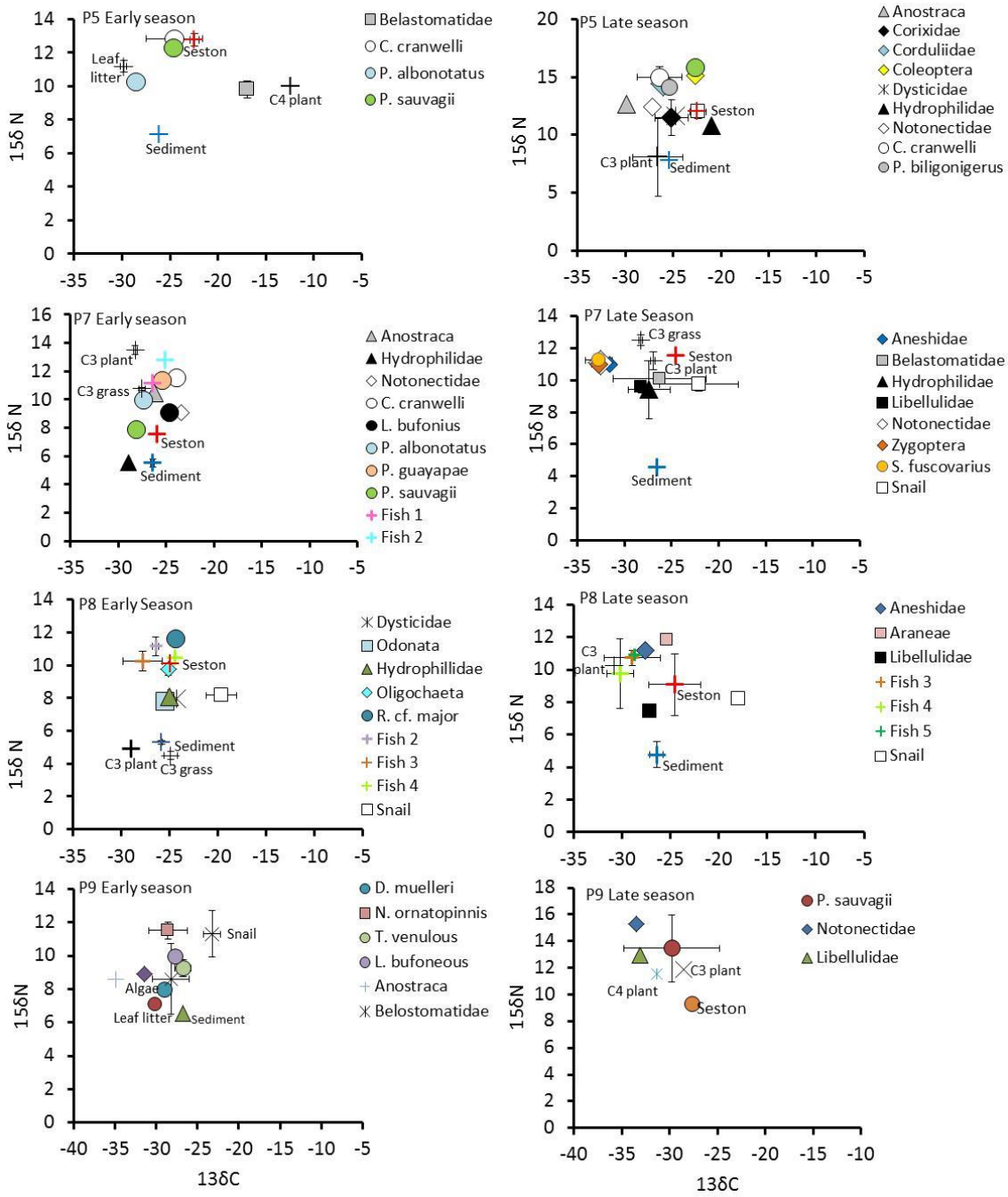
2855, 5663, 5665, 5703, 5666, 5664, 5661, 5702, 5662, 5660, 5779, 5860, 5863, 5864, 1373, 1374, 1375, 1376, 1377, 1788, 1774, 1780, 1371, 1776, 1793, 1790, 1372, 1787, 1792, 1783, 1786, 1789, 4890, 2489, 2715, 2496, 1779; *L. fuscus*: 6345, 6346, 4548, 8897, 4816, 4817, 3516, 8525, 3814, 5248, 2887, 675, 669, 491, 307, 2863, 3089, 4910, 4968, 3065, 138, 283, 137, 7906, 305, 395, 6341a, 6340, 6339, 6338, 6341b; *L. labyrinthicus*: 5650, 5247, 2990, 1291, 1292, 4426, 6336, 88, 3081, 3527, 5648, 5646, 5647, 5649; *L. laevis*: 5869; *L. laticeps*: 4240, 4241; *L. latinasus*: 4723, 4737, 4717, 4716, 5697, 5695, 5696, 5699, 5698, 5700, 8522, 8479, 8496, 8505, 8478, 4729, 4724, 4736, 4728, 4726, 4727, 4722, 4725, 5481, 5480, 5479; *L. leptodactyloides*: 6295, 8915, 8916, 8787, 4059, 6694, 1861, 3648, 3649, 2916, 1945, 1748, 8519, 8500, 8509, 8497, 2034, 2098, 2031, 883, 1865, 1399, 3844, 3845, 1399, 1839, 1836, 1848, 1845, 1844, 1847, 1843, 1845, 1834, 1840, 1842, 1841, 1848, 1584, 1160, 1158, 1154, 1155, 1159, 1752, 1753, 1751, 1754, 2589, 2588, 2602, 2033, 4838, 4839, 4399, 4480, 4479, 4478, 5523, 3105, 3073, 3104, 3074, 3111, 3125, 3088, 3091, 3112, 3994, 3980, 3995, 5750, 5749, 767, 768, 734, 737, 5048, 5017, 6128, 1537, 1533, 1529; *L. llanensis*: 4242, 4243, 7600; *L. mystacinus*: 3279, 4792, 4878, 4876, 4875, 9198, 4681, 4879, 4874, 4877, 4880, 1933, 5634, 5635, 3069, 3067, 3061; *O. americanus*: 4699. 4701, 9262, 1989, 1509, 4669, 4903, 5539, 7754, 7755, 7760; *P. albonotatus*: 10740, 1965, 5783, 5895, 5953, 5638, 5639, 5841, 5988, 5989, 5990, 5991, 5992, 2298, 2299, 4685, 4689, 1176, 1178, 1180, 9, 6232, 6233, 6234, 5893, 5600, 5524, 5527, 5528, 1561, 1563, 1564, 1565, 75, 5839, 1056, 3264, 3265, 3277, 3278, 4926, 4999, 5000, 3313, 3310, 1048, 2379, 2494, 2576, 2599, 2598, 2600, 2601, 2623, 2626, 5500, 5501, 5502, 5498, 5499, 3343, 655, 656, 664, 665; *P. biligonigerus*: 4769, 1575, 4829, 4767, 4485, 4486, 5998, 5838, 175, 396, 2850, 5475, 7490, 5652, 3382, 5241, 5514, 5515, 5525, 5526, 3263, 3280, 3281, 4843, 8513, 8511, 8498, 8512, 8510, 8524, 8477, 5785, 5821, 5803; *P. boliviana*: 7528, 7924, 4674, 9163, 9162; *P. cuvieri*: 3312, 3314, 3311, 7443, 7444, 983, 6349, 6352, 6348, 6351, 6347, 6350; *P. guayapae*: 10276, 10279, 10280, 10274, 10282, 10273, 10271, 10277, 10268, 10283, 10275, 10266, 10281, 10270, 10269, 10284, 10267, 10265, 10272, 10278, 10293, 10296, 10287, 10291, 10295, 10302, 10290, 10289, 10288, 10304, 10300, 10299, 10294, 10298, 10303, 10286, 10292, 10285, 10297. 10301, 3250, 3252, 3245, 3249, 3246, 3248, 3247, 3251, 3244; *P. hypochondrialis*: 5513, 5512, 4977, 4772, 4506, 4813, 80, 2355, 2409, 1944, 7632, 7579, 7630, 2399, 2400, 2397, 2274, 2398; *P. paradoxa*: 2241, 2244, 2246, 2240, 2245, 2242, 2411, 2243, 2317, 4906, 8753, 8490, 2885, 3268, 306, 2727, 1732, 1738, 441, 580, 2248, 2384, 2291, 2318, 2247, 3412, 5507, 5503, 5505, 5508, 1485, 1486; *P. sauvagii*: 4644, 4720, 3564, 4671, 4673, 7572, 7576, 5255, 4676, 4677, 1983, 1984, 5623, 5624, 5625, 4672, 7533, 7563, 7564; *R. major*: 4732, 4928, 5808, 4721, 8474, 8473, 2744, 3098, 4, 5686, 5684, 5685, 7937, 4731, 5561, 149, 2; *R. schneideri*: 10746, 7618, 7804, 7757, 7758, 7759, 1, 8607, 2264, 2265, 3076, 5772, 5996, 154, 155, 839, 2877, 2878, 2879, 2880, 2881, 2882, 2201, 2226, 2491, 205, 206, 207, 208, 218, 5994, 156, 157, 1911, 1912, 1913, 1921, 1922, 1923, 7834, 1975, 7376, 5258, 3000, 999, 6344, 6358, 3548, 3496, 3488, 6579, 232, 234, 241, 242, 3841, 548, 550, 551, 5601, 5602, 5603, 2130, 5896, 8910, 8911, 171, 8718, 8719, 691, 7724, 2907, 5768, 5769, 1567, 1301, 5599, 1302, 2266, 2263, 2696, 2604, 2697, 2695, 2683, 3834, 2490, 2297,

3416, 3417; *S. acuminatus*: 7956, 7960, 7959, 7955, 7957; *S. fuscovarius*: 4678, 1978, 9155, 1980, 4662, 7, 4653, 8488, 8515, 681, 5521, 5533, 858, 85, 9269, 4491, 4802, 1986, 5534, 5535, 5536, 5537, 5538, 9235, 9167, 2921, 2922, 2923, 4776, 4778, 4779, 4780, 5629, 5630, 5631, 5632, 3031, 3033, 3084, 3092, 3120, 7481, 7530, 7546, 7529, 7532, 7323, 720, 752, 753, 589; *S. nasicus*: 5626, 5628, 5627, 4843, 3117, 3107, 7561, 7554, 7567, 6756, 7573, 879, 568, 4507, 3114, 3115, 3095, 3116, 7577, 6237, 6238, 8523, 8493, 219, 222, 220, 221, 4679, 160, 159, 158, 2668, 2667, 2669, 2672, 124, 4650, 4649, 3275, 3276, 3259, 3260, 4777, 3159, 125, 121, 3041, 3042, 4775, 3393, 3394, 185, 202, 6435, 6433, 6432, 6434, 5250, 6405, 6407, 6403, 6404, 4766, 4769, 4771, 4770, 4768; *T. typhonius*: 2997, 2920, 262, 5249, 4630, 4631, 5777, 249, 183, 6687, 6688, 2767, 2768, 254, 2036, 5553, 4905, 4904, 6387, 5563, 868, 5693, 4659, 268, 30, 2213, 2213, 2369, 3231, 3232, 7525, 7589, 7580, 7524, 7660, 7659, 4648, 450, 4010, 4011, 8517, 265, 3293, 3078, 3063, 3066, 984, 101, 1723, 1720, 1718, 1721, 1719, 1726, 1727, 1725, 5773, 217, 247, 3282, 3266, 3255, 3420, 4487, 29, 2864, 2866, 2867, 2865, 2875, 2870, 2869, 2871, 2868.

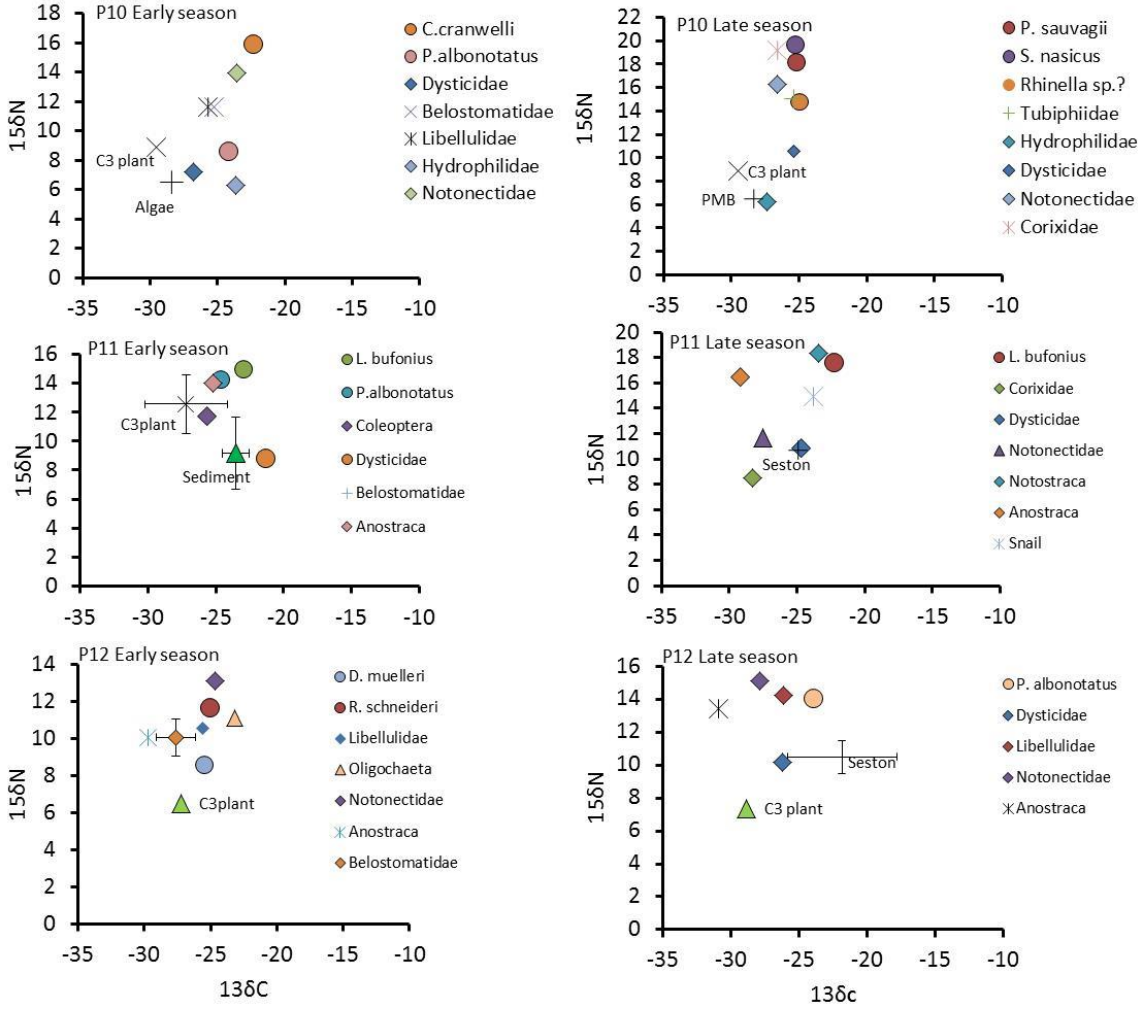
Appendix C. Bi-plots of ^{13}C and ^{15}N for tadpoles, aquatic invertebrates, and three basal sources for the remaining ponds surveyed in the study area. Isotope ratios were compared for certain taxa that occurred near the beginning (early season) and end (late season) of the annual rainy season. The bi-plot for pond 6 are depicted in Fig. X.



Appendix C. Continued.



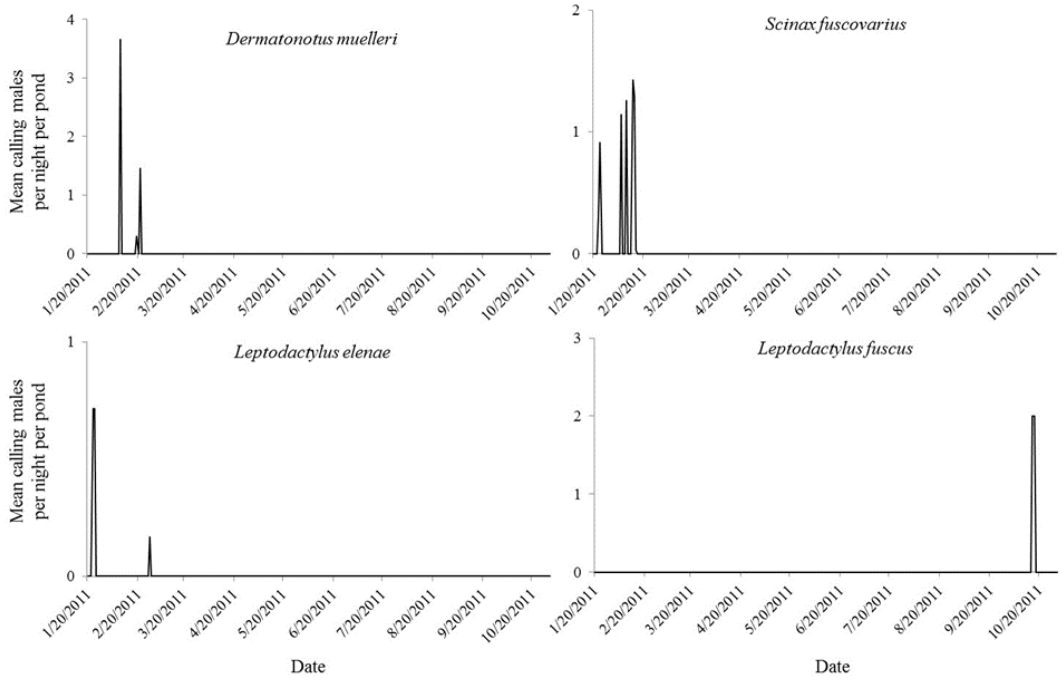
Appendix C. Continued.



Appendix D. Occurrence of calling males across seven ponds surveyed in the Bolivian Gran Chaco. The x symbol indicates that the species was observed calling at least once at the pond indicated. Blank cells represent no calling detected at that pond for a given species.

Family / Species	Pond 1	2	3	4	5	6	7
Bufonidae							
Rhinella major (Müller & Hellmich. 1936)	x	x	x	x	x	x	x
Rhinella schneideri (Werner, 1894)	x	x	x	x	x	x	x
Ceratophryidae							
Ceratophrys cranwelli (Barrio, 1980)	x	x	x	x	x	x	x
Hylidae							
Phyllomedusa sauvagii (Boulenger, 1882)	x	x	x	x	x	x	x
Scinax fuscovarius (Lutz, 1925)	x	x				x	
Scinax nasicus (Cope, 1862)	x	x	x	x	x	x	x
Leptodactylidae							
Leptodactylus bufonius (Boulenger, 1894)	x	x	x	x	x	x	
Leptodactylus elenae (Heyer, 1978)							x
Leptodactylus fuscus (Schneider, 1799)	x						
Physalaemus albonotatus (Steindachner, 1864)	x	x	x	x	x	x	x
Physalaemus biligonigerus (Cope, 1861)	x	x	x	x	x	x	x
Pleurodema guayapae (Barrio, 1964)	x	x	x	x	x	x	
Microhylidae							
Dermatonotus muelleri (Boettger, 1885)					x		x
Odontophryidae							
Odontophrynus americanus (Duméril & Bibron, 1841)	x	x	x	x	x	x	x

Appendix E. Call intensity scores for four species of anurans each night from 20 January 2011 – 31 October 2011: *Dermatonotus muelleri*, *Scinax fuscovarius*, *Leptodactylus elenae*, and *Leptodactylus fuscus*. Ponds were located within the vicinity of the Isoceño community of Yapiroa, Cordillera Province, Santa Cruz Department, Bolivia. These species were detected less than 10 days during the survey period and excluded from analyses. Note the differences in the value range on the y-axis.



Appendix F. Specimens examined are from Museu Nacional Rio de Janeiro (MNRJ) and the University of Kansas Natural History Museum and Biodiversity Institute (KU). Institution abbreviations follow Sabaj Pérez (2013).
Ceratophrys aurita—BRAZIL: Bahia: Nova Viçosa (MNRJ 19030–19031); Espirito Santo: Sooretema, Linhares (KU 92740)
Ceratophrys calcarata— COLOMBIA: Bolivar: Alto de Quimari, 500 m (KU 144966); VENEZUELA: Amazonas: Puerto Ayacucho, 110 m (KU 207528).
Ceratophrys cranwelli —BOLIVIA: Santa Cruz: Parapati (KU 92741); PARAGUAY: Central: Asuncion (KU 145088).
Ceratophrys ornata— ARGENTINA: Buenos Aires: Olazabal, Tuzaingo (KU 175560); Buenos Aires: Necochea (KU 186864).
Chacophrys perottii —ARGENTINA: Cordoba: Totoralejos (KU 128846–51, 191927–28, 191932).
Lepidobatrachus asper—ARGENTINA: Santiago del Estero: Santiago del Estero (KU 80782); PARAGUAY: Central: Asuncion (KU 145089).
Lepidobatrachus laevis—ARGENTINA: Formosa: Formosa (KU 128853).